The Susceptibility of Hybrid Eucalypts to Pests

by

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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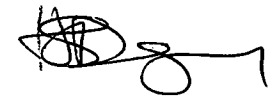

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Abstract

Interspecific hybridisation is potentially a very useful tool for tree breeders, particularly as a source of variation for important genetic traits. However, hybrids in several tree species have been shown to be highly susceptible to damage by some pests. Therefore, the primary aim of this study was to determine if hybrids in *Eucalyptus* were more susceptible to pests than pure species. To achieve this, the responses of a number of vertebrate, invertebrate and fungal pests to hybrid eucalypts and their parent species, were examined in several experimental field trials and in a natural hybrid zone.

Firstly, susceptibility of hybrids to the fungal leaf disease caused by *Mycosphaerella* spp. was examined in an experimental field trial, including first generation (F_1) hybrids and controlled crosses of *E. globulus* and *E. nitens*. Both the host preference and genetic variation in resistance were investigated. Heritabilites were low to moderate and disease severity was greatest on the F_1 hybrids. Hybrids were more susceptible to damage by *Mycosphaerella* than both *E. globulus* and *E. nitens*.

Secondly, the host species preference and hybrid susceptibility was examined for chrysomelid leaf beetles (*Chrysophtharta* spp. and *Paropsis* spp.), the gum leaf skeletoniser (*Uraba lugens*) and for brush tail possums (*Trichosurus vulpecula*) in a number of experimental hybrid trials. Heritability estimates were very low for possum damage on *E. globulus*, and high for *E. nitens*. The proportion of dominance variation for possum damage was consistantly higher than heritability estimates in *E. globulus,* and at least as high as heritability estimates in *E. nitens*. *E. morrisbyi* and *E. gunnii* consistently had the most possum damage and *E. globulus, E. nitens* and *E. johnstonii* the least. Responses of the pest species to the different hosts and hybrids varied but hybrids were generally intermediate in their susceptibility to the different herbivores when compared with their parent species.

Thirdly, this thesis examines the host species preference of a number of insect taxa and the host susceptibility of *E. amygdalina, E. risdonii* and *E. amygdalina x E. risdonii* hybrids. In a previous study by Whitham *et al.* (1995) in a natural hybrid zone, hybrids were found to be more susceptible to both insect and fungal taxa than either *E. amygdalina* or *E. risdonii* . The genetic basis of this observed hybrid susceptibility was examined by determining the distribution of a number of insect taxa in an experimental field trial, where the pedigree of the majority of hybrids and pure species was known. Species richness was found to be greater on hybrids than pure parent species. Furthermore, F_1 eucalypt hybrids tended to be more susceptible to attack than advanced generation hybrids, arguing against hybrid breakdown being the

cause of the greater susceptibility of the hybrids. Mechanisms contributing to the observed responses on the *E. amygdalina x E. risdonii* hybrids were also examined. Leaf toughness and the oil content and composition of the parent species and hybrids was determined and the effect of the different oil components on the distribution of the insect taxa was discussed.

Finally, the cause of preferential weevil damage on hybrids between *E. amygdalina* and *E. risdonii* in a field trial was examined. While other factors such as larval survival and egg loss were examined, oviposition by the eucalypt weevil *Gonipterus scutellatus* was highly biased towards the hybrid phenotypes. This lead to much larger larval numbers on hybrids and was therefore the primary factor contributing to the higher damage observed on hybrids in the field trial.

Results are discussed in terms of the susceptibility of hybrids and their usefulness in temperate hardwood forestry. The possible nature of hybrid susceptibility in *Eucalyptus* is also examined.

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Table of Contents

Chapter 1

Hybrids, hybrid susceptibility and pests of *Eucalyptus*

Hybrid susceptibility

There is considerable interest in the use of interspecific hybrids for tree breeding in *Eucalyptus* (Potts and Potts 1986; Campinhos and Ikemori 1989; Volker 1995), and in other genera (e.g. *Larix*, Li and Wyckoff 1994; *Pinus*, Nikles 1991, Blada 1994; *Populus*, Hsiang and Chastagner 1993, Wu 1994a, Wu 1994b, Rajora *et al.* 1994). This interest is due primarily to the possibility of exploiting gain from heterosis, (Klekowski 1988, Bos and Sparnaaij 1993), introgression of new genetic material and combining genes from species with complementary traits (Martin 1989, Nikles 1991, Dieters *et al.* 1989). In some cases hybrids have been shown to have improved resistance to pests (e.g. canker resistance in *E. grandis x E. urophylla* hybrids, Campinhos and Ikemori 1989). However, several recent studies on trees suggest that natural hybrids may be more susceptible to pests than pure parental types (Drake 1981, Whitham 1989, Whitham *et al.* 1994). If this susceptibility has a genetic basis, and is a general phenomenon, then the use of hybrids in tree breeding programs may have important long term implications to forestry.

The first indications that natural hybrids may be more susceptible to pests, resulted from investigations in a natural hybrid zone of *Populus* (Whitham 1989). The aphid *Pemphigus betae*, was found to be concentrated within the *Populus* hybrid zone and on the hybrid phenotypes within the hybrid zone (Whitham 1989, Paige *et al.* 1990). Whitham (1989) postulated from this study that hybrids were genetically more susceptible to pests than pure parent species and may be acting as both ecological and evolutionary sinks for pests. Hybrid susceptibility has subsequently been investigated in a number of natural hybrid zones between tree species, with results both conflicting and supporting this concept. Recent reviews in this area, include (Fritz *et al.* 1994, Strauss 1994, Arnold and Hodges 1995, Haukioja, *et al.* 1994). It is necessary for completeness, that some of the information reviewed, particularly by (Fritz *et al.* 1994, Strauss 1994) be re-reviewed in this thesis.

In species other than trees, the majority of studies suggest that hybrids are more susceptible to pests than pure parent species. Hybrid fish (cyprinids), were found to be both highly susceptible to gill parasites (Dupont and Crivelli 1988) and in a separate hybrid zone, to a cyprinid parasite (Le Brun *et al.* 1992). In a hybrid zone between two different mice species, hybrids carried greater loads of parasites than adjacent pure species (Sage *et al.* 1986). In contrast, lice were preferentially

distributed on pure species of pocket gophers, rather than on the hybrid, suggesting hybrid superiority (Heaney and Timm 1985).

Hybridisation and the fitness of hybrids in tree species has recently received considerable attention. In particular, the susceptibility of hybrids to pests when compared with pure parent species has been examined (Whitham 1989, Boecklen and Spellenberg 1990, Whitham *et al.* 1991a, Whitham *et al.* 1991b, Hanhimäki *et al.* 1994, Whitham *et al.* 1994). While the majority of these studies have been undertaken in natural hybrid zones, e.g.Whitham (1989), Boecklen and Spellenberg (1990), Whitham *et al.* (1991), Hanhimäki *et al.* (1994), Whitham *et al.* (1994), they are still valuable in the general assessment of hybrid susceptibility.

Studies of tree hybrids where hybrids were found to have intermediate susceptibility or greater resistance when compared with parents species included a leaf mining moth *Phyllonorycter* sp. in a *Quercus* hybrid zone (Preszler and Boecklen 1994). The moth was distributed preferentially on one of the host parent species, decreasing in density through the categories of hybrids to the lowest density on *Q. grisea*. However, on the same hybrids, gall forming wasps were also distributed preferentially on *Q. gambelii*, although there were individual gall responses where the hybrid appeared to be more susceptible (Boecklen and Larson 1994). Boecklen and Spellenberg (1990) and Aguilar and Boecklen (1992) found a reasonably equitable distribution of insect guilds across hybrids and pure species in a *Quercus* hybrid zone. Similarly, Christensen *et al.* (1995) had conflicting reports with some insect species preferring the *Pinus* hybrids and others preferring the pure parent species.

In the same hybrid zone investigated by Whitham (1989), folivory was found to be highest on hybrid *Populus* (33%) when compared with pure parents (25% and 16.5%) (Floate and Whitham 1994). Hybrid palo verde trees (*Cercidium floridum* and *C. microphyllum*) were less resistant to attack by bruchnid beetles than either parent species, due mainly to a lack of seed coat resistance and early pod abscission (Siemens *et al.* 1994). Fritz *et al.* (1994) has documented a wide range of responses of natural *Salix* hybrids (*S. sericea x S. eriocephala*), including hybrid susceptibility, to pests.

The investigation of hybrids has been taken to higher trophic levels. Hybrids have been shown to support a greater number of birds than adjacent pure species in Whitham's (1989) natural *Populus* inter-specific hybrid zone (Martinsen and Whitham 1993). Parasitism of a leaf mining moth on *Quercus* hybrids was not however, concentrated on hybrid plant phenotypes but on parent species

(Boecklen and Spellenberg 1990). However this could also be interpreted as the greater success of the moth on the hybrids because of the lower parasitism present.

Susceptibility in Eucalyptus

The genus *Eucalyptus* is renowned for its propensity for hybridisation (Griffin and Cotterill 1988) and studies of three different natural hybrid zones of *Eucalyptus* have shown that in general, natural hybrids do support greater insect loads and species richness than pure phenotypes (Morrow *et al.* 1994, Whitham *et al.* 1994). In the case of hybridisation between *E. risdonii* x *E. amygdalina,* 73% of the 40 taxa examined were significantly more abundant in the hybrid zone than in adjacent pure species zones, and 5 of the 40 species were largely restricted to the hybrid zone. Species richness (the number of insect taxa per tree), increased both in the hybrid zone and on the hybrids within the hybrid zone. Whitham *et al.* (1994) suggested that the increased species richness on the hybrid phenotypes within the hybrid zone could be due to (i) increased genetic susceptibility of the hybrids or, (ii) increased stress at the distributional limits of species increasing susceptibility to attack. Genetic susceptibility is explained by the breakdown of multigenic resistance (Whitham *et al.* 1994). Both simple and multigenic variation to resistance in plants can occur (Fritz and Simms 1992, Kennedy and Barbour 1992). Through the process of hybridisation, simple single gene resistance can be either excluded or included and resistance that is multigenic is likely to be disrupted (Grant 1981, Morrow *et al.* 1994). However, this theory of hybrid breakdown is yet to be experimentally validated.

Whitham *et al.* (1994) also suggests that the increased species richness and abundance observed on pure *E. risdonii* and *E. amygdalina* phenotypes in a natural hybrid zone may be explained by at least 5 mechanisms acting singly or in concert. Firstly, insects may overflow from hybrids with high herbivore loads to nearby parental types. Secondly, the close proximity of the different plant species with different insect faunas could facilitate the mixing of these faunas. Thirdly, increased stress at species boundaries may make both parental species more attractive to herbivores. Fourth, expanded leafing-out phenologies in a hybrid zone could expand the season that trees can be successfully utilized by herbivores. Fifth, trees classified as pure phenotypes in the hybrid zone may actually be complex advanced generation hybrids which can be detected by insects but not by the taxonomic methods used (Whitham *et al.* 1994).

Pests on eucalypts

In Australia, eucalypts have a wide range of both insect and fungal pests (e.g. Marks *et al.* 1982, Elliot and deLittle 1984, Howes 1990). With the continued domestication

of eucalypts for forestry purposes, the effect of pests will become increasingly important (Abbott 1993b). Certainly, with the prospect of genetic improvement, hybridisation may become important as a source of new genetic variation (Zobel and Talbert 1984). However, a more thorough understanding of the biology of important pests is also necessary for long-term plantation success in addition to the use of genetic resources through breeding (Abbott 1993b). With this in mind, a general review of major insect and fungal forest pests in temperate Australia follows.

Insects:

The major insect pests in eucalypt plantations have been reviewed for each state of Australia: Tasmania (Bashford 1993), Victoria (Neumann 1993), South Australia (Phillips 1993), Western Australia (Abbott 1993a), New South Wales (Stone 1993), and Queensland (Wylie and Peters 1993). The most widespread and damaging pests include *Uraba lugens, Mnesampela privata, Phaulacridium vittatum, Chrysophtharta* spp., *Anoplognathus* spp. and *Perga* spp. There is little detailed information on pests of commercial eucalypt plantations (eg Mazanec 1985, Leon 1989, Bennet *et al.* 1992, Candy *et al.* 1992, Edwards *et al.* 1993, Neumann 1993), particularly with respect to the genetics of resistance (Ohmart *et al.* 1984, Farrow *et al.* 1994, Floyd *et al.* 1994, Raymond 1995). A brief summary of some of the important insect pests follows. These brief summaries will be heavily biased towards pests that are important in eucalypt plantations in Australia.

Mnesampela privata (Autumn gum moth)

M. privata has one generation per year (univoltine). Pupation occurs from five to seven months in cocoons in the soil (Elliot and Bashford 1978, Neumann 1993), In SW Australia, larvae are present through autumn, winter and spring. *M. privata* larvae preferentially attack the glaucous juvenile foliage in young plantations before development of the adult foliage. In southern Tasmania, most defoliation occurs in autumn and winter (Elliot and Bashford 1978). *M. privata* has caused serious damage in eucalypt plantations, and can cause complete defoliation (Farrow *et al.* 1994, Floyd *et al.* 1994). Trees usually recover from light to moderate damage, but if successive damage occurs across several seasons, death will often occur (Elliot and Bashford 1978). Although *M. privata* has a wide host range, it is particularly a problem on *E. globulus, E. nitens* and *E. delegatensis* in Tasmania (Elliot and Bashford 1978, Elliot and deLittle 1984). *M. privata* also attacks *E. viminalis* and is a pest in eucalypt nurseries in South Australia (Moore 1963, Carne and Taylor 1984). Further details of this pest can be found in Elliot and deLittle (1984) and McQuillan (1985).

Uraba lugens (Gum leaf skeletoniser)

U. lugens is univoltine in Tasmania but may be bivoltine, particularly in warmer areas of mainland Australia (Campbell 1962, Elliot and deLittle 1984, Howes 1990). There are 11 instars and after the fifth instar, the larvae retain the head capsules. Larvae are gregarious until the fourth or fifth instar, after which they tend to be more solitary feeders (Campbell 1962). Pupation occurs in silken cocoons, usually in the leaf litter (Campbell 1962, Howes 1990). *U. lugens* is a problem predominantly on *E. camaldulensis, E. globulus, E. obliqua, E. delegatensis, E. regnans, E. viminalis, E. blakelyi, E. marginata* and *E. wandoo* (Campbell 1962, Harris 1974, Harris 1975, Carne and Taylor 1984, Howes 1990, Floyd *et al.* 1994). It is a persistent but low level pest of plantations and is more of a problem for trees planted as windbreaks in rural areas (Harris 1975, Howes 1990), although outbreaks causing widespread defoliation have been recorded (Campbell 1962). Generally, *U. lugens* does not kill mature trees although attack on young trees can be severe enough to kill them (Elliot and deLittle 1984). Further details on this pest are given in (Brimblecombe 1962, Morgan and Cobbinah 1977, Cobbinah 1985, Allen 1990).

Chrysophtharta spp. (eucalypt leaf beetles)

Chrysophtharta bimaculata is the main pest species on eucalypts in Tasmania (Kile 1974, Raymond 1995). This species is suspected to be both univoltine (NW Tasmania) and bivoltine (southern forests of Tasmania) (see Greaves 1966, deLittle 1983). *C. bimaculata* has four larval instars, but it is the third and fourth instars which cause the most damage (Greaves 1966). Its preferred host species is *E. regnans*, although it will feed on other species, including *E. delegatensis* and *E. nitens* (see deLittle and Madden 1975, deLittle 1982, deLittle 1989, Li 1993). *C. bimaculata* has caused a severe loss of production in *E. regnans* (Greaves 1966, Leon 1989, Candy *et al.* 1992, Cremer 1972, Elliot *et al.* 1993). Damage caused by this beetle has been shown to be highly negatively correlated with growth (Raymond 1995). While some research is now directed at a fully integrated pest management (IPM) system, particularly with the use of *Bacillus thuringensis* (Bt), extensive chemical control methods are still employed (deLittle 1989, Elliot *et al.* 1992). It is likely, with the extensive planting of one species of eucalypt throughout Tasmania (*E. nitens*), that this pest will become increasingly economically important for future wood production (Candy *et al.* 1992, Elliot *et al.* 1992, Abbott 1993b). However, the fact that significant genetic variation was found in both *E. regnans* and *E. nitens* for defoliation caused by *C. bimaculata* (Raymond 1995) suggests that breeding for resistance may play a role in intensive forestry in the future. Further biological information can be found in Elliot and de Little (1980) and deLittle *et al.* (1990).

Other chrysomelid beetles (*Paropsis, Chrysophtharta*).

On the mainland of Australia, chrysomelid beetles have at least two generations per year. Eggs are usually laid in early summer and beetles can be found from October to early March (Howes 1990). Larvae are generally gregarious and attack a wide range of eucalypt species (Carne 1966, Carne and Taylor 1984, Cameron *et al.* 1989). Adult beetles do not generally cause severe damage to individual trees (Howes 1990). The levels of damage caused by other chrysomelids to eucalypts (Fox and Macauley 1977, Ohmart *et al.* 1985, deLittle 1989), likely host resistance mechanisms (Edwards 1982b) and the biology or ecology of some chrysomelids is also available in the literature (Cumpston 1939, Strauss and Morrow 1988).

Phaulacridium vittatum (Wingless grasshopper)

P. vittatum is univoltine. Eggs are laid from late summer to early autumn and the insect diapauses over winter as embryos (Howes 1990, Neumann 1993). Nymphs hatch and are active during spring and begin to mature from November onwards (Howes 1990). When mature, the grasshoppers are able to climb and it is only after this stage that damage to trees occurs. *P. vittatum* is a pest of improved pastures in New South Wales, South Australia and Victoria (Abbott 1993a, Neumann 1993, Stone 1993, Bailey *et al.* 1994, Baker *et al.* 1994, Milner *et al.* 1994). While *P. vittatum* can cause damage to plantations (e.g. Abbott 1993a), it is more a pest of shelterbelts in rural areas (Howes 1990). Damage is greatest in times of drought, when the primary host of the grasshoppers is unavailable (Howes 1990, Abbott 1993a, Neumann 1993). Attack can be severe enough to cause death and damage to seedlings and can cause high levels of host mortality (Howes 1990). Further biological information on this pest can be found in Clark (1967), Baker (1988) and Baker *et al.* (1994).

Anoplognathus *sp. (Christmas beetles)*

Christmas beetles are univoltine or hemi-voltine (one generation every 2 years). Eggs are laid in the soil and larvae feed in the soil until pupation occurs (Neumann 1993). Adults over winter and emerge in spring to summer. Damage to trees is caused only by adults (Neumann 1993, Stone 1993). The species which cause the most damage are *A. chloropyrus* and *A. porosus* although *A. porosus* is less likely to cause significant loss of growth (Carne *et al.* 1974, Stone 1993). Damage is greatest when young plantations are adjacent to rural land (Pryor *et al.* 1968, Carne *et al.* 1974, Carne and Taylor 1984). Leaves affected by Christmas beetles have a serrated appearance and the upper tree crown is often stripped of leaves giving a characteristic 'brooming' effect (Neumann 1993). Preferred host species include *Eucalyptus botryoides, E. globulus* and *E. grandis*, although *E. fastigata, E. obliqua* and *E. regnans* are also attacked (Neumann 1993).

Perga spp. (sawfly larvae)

Perga larvae are usually black or brown, are covered sparsely with white bristly hairs and are gregarious during the day, dispersing at night to feed (Howes 1990). When disturbed, these larvae often 'spit' an odorous fluid (see Morrow *et al.* 1976). Larvae are present during late winter and spring and pupate in the soil in spring (Elliot and deLittle 1984, Howes 1990). Pupation usually lasts two to three years. *Perga affinis* is the most studied of the sawflies (Carne 1962, Carne 1965, Carne 1969). It is highly gregarious and a voracious feeder, particularly in the last two of its six instars (Carne 1962). Feeding behaviour of this pest and the possible sabotage of host defences through leaf petiole chewing is examined by Weinstein (1990). More specific life cycle information on *Perga affinis* is given in Reik (1961) and Tait (1962). Host range for *Perga* spp. includes *E. camaldulensis, E. melliodora, E. blakelyi* and *E. wandoo* (Carne 1962, Howes 1990). Serious defoliation is usually rare and without repeated severe attacks, death from this pest species is unlikely.

Pathogens

The effect that pathogens have on the productivity of plantations is not clearly understood. While there are a large number of pathogens in Australian native forests (see Heather 1967a, Neumann and Marks 1976, Heather and Griffin 1984, Withers *et al.* 1994), there has not been a great deal of research in this area. There are a number of reviews of the disease status of Australian forests (e.g. Heather 1967a, Gibson 1975, Neumann and Marks 1976, Heather and Griffin 1984, Old 1990, Withers *et al.* 1994). However, it is mainly through the pest status of certain pathogens in other countries that a number of useful reports have been produced (Heather 1971, Lundquist 1985, Lundquist and Purnell 1987, Crous *et al.* 1989b, Crous *et al.* 1989c, Wingfield and Swart 1994). In short, there is still a large amount of work to do in this area and only a few important species are well documented. The species that are currently recognised as a potential problem in plantation forestry and that have a reasonable base in the literature, are outlined below.

Mycosphaerella *(crinkle leaf)*

Mycosphaerella one of the most important pathogens to the eucalypt forest industry. It has been shown to cause severe defoliation both on mature trees and on seedlings in the nursery (e.g. Marks 1979a, Wilcox 1982a, Wilcox 1982b, Lundquist and Purnell 1987, Carnegie *et al.* 1994). This pathogen causes leaf spot, ultimately reducing the level of effective photosynthetic area (Dick and Gadgil 1983, Carnegie *et al.* 1994). Levels of damage have been found to be highly negatively correlated with growth at the provenance level (in *Eucalyptus globulus*; Carnegie *et al.* 1994). *Mycosphaerella* has been relatively well studied on eucalypts, with over 10 species now known to

cause leaf disease (see Park and Keane 1982a, Park and Keane 1982b, Park and Keane 1984, Park and Keane 1987, Park 1988a, Park 1988b, Carnegie 1991, Crous *et al.* 1991, Crous *et al.* 1993a, Crous *et al.* 1993b, Carnegie and Keane 1994, Crous and Alfenas 1995). Of all the *Mycosphaerella* species, the most damaging in forestry include *M. molleriana* (Thüm) Lindau (syn. *M. nubilosa* (Cke.) Hansf., (see Crous *et al.* 1991) and *M. cryptica* (Cooke) Hansf. (Marks 1979a). *M. cryptica* has a wide host range and is able to infect both juvenile and adult foliage (Park and Keane 1982b). In Australia, *M. molleriana* has been described only from the juvenile foliage of *E. globulus, E. cypellocarpa* and *E. bridgesiana* (Park and Keane 1982b). However, in South Africa, *M. molleriana* is reported to have a much wider host range including *E. nitens, E. grandis, E. saligna* and *E. viminalis* (Crous *et al.* 1989b). As a direct result of this, there is some debate over the synonym status of *M. molleriana* and *M. nubilosa* (Carnegie pers. comm.). While *Mycosphaerella* is a problem in Australia (Marks 1979a, Marks *et al.* 1982, Carnegie *et al.* 1994), in South Africa this disease has in some cases caused plantation areas to be abandoned (Lundquist and Purnell 1987). For further information on this pathogen is given in Chapter 2.

Other leaf diseases

Other important pathogens of *Eucalyptus* leaves include *Phaeseptoria eucalypti, Aulographina eucalypti, Cercospora* spp. *and Seimatosporium* spp. (Heather and Griffin 1984, Crous *et al.* 1989b, Crous *et al.* 1989c, Old 1990). *Phaeseptoria eucalypti* (syn. *Hendersonia grandispora*) is a leaf spot fungus with a wide host range (see Heather 1967a, Heather 1967b, Dick 1982, Swart and Walker 1988 Crous *et al.* 1989a, Crous *et al.* 1989c). It can cause severe damage to seedlings but it is unlikely to defoliate mature trees (Sharma *et al.* 1984, Crous *et al.* 1989b, Crous *et al.* 1989c). *Aulographina eucalypti* causes corky leaf spots on *Eucalyptus* leaves (see Heather 1971, Keane *et al.* 1981, Dick 1982, Marks *et al.* 1982, Sharma *et al.* 1984, Wall and Keane 1984, Park 1988a, Crous *et al.* 1989b, Crous *et al.* 1989c). It also has a wide host range and can cause extensive defoliation (Crous *et al.* 1989c). *Cercospora* spp., including *C. epicoccoides* and *C. eucalypti,* cause leaf spotting (Crous *et al.* 1989b, Dick 1982, Keane *et al.* 1981, Neumann and Marks 1976). The host range includes some of the more common commercial eucalypt species *E. globulus, E. nitens, E. delegatensis,* and *E. regnans* (Dick 1982, Crous *et al.* 1989b). *Seimatosporium* spp. is also found on a number of commercial eucalypts, including *E. globulus, E. nitens, E. regnans, E. delegatensis* and *E. dives* (Crous *et al.* 1989b). *Seimatosporium* spp. causes leaf spots on its host, which are usually angular in shape (Marks *et al.* 1982, Crous *et al.* 1989b). Other leaf diseases are documented in Gibson (1975), Carne and Taylor (1984), Crous *et al.* (1989b) and Crous *et al.* (1989c).

Phytophthora

Phytophthora causes root rot in a wide range of tree species (see Gibson 1975, Old 1979, Marks *et al.* 1982, Erwin *et al.* 1983, Marks and Smith 1991, Cahill 1994). *Phytophthora* infects its host primarily via motile spores in the soil (Irwin *et al.* 1995). A detailed life cycle, for both sexual and asexual stages can be found in Podger and Batini (1971), Newhook and Podger (1972), Podger (1972), Neumann and Marks (1976), Weste and Marks (1987), Marks and Smith (1991), Irwin *et al.* (1995). In *Eucalyptus*, *P. cinnamomi* is one of the primary causes of root disease, although *P. atricola* and *P. megasperma* are also implicated (Old 1979, Cahill 1994). Although *P. cinnamomi* has a wide host range in eucalypts, including *E. calophylla, E. fraxinoides, E. sieberi, E. viminalis, E. globulus, E. fastigata, E. smithii, E. elata,* it is mainly a problem in the *E. marginata* (jarrah) forests of Western Australia (Marks 1979b, Cahill *et al.* 1992, Cahill 1994, Irwin *et al.* 1995). *Phytophthora* has been identified as a primary cause of dieback in Australia (see Marks 1979b, Cahill *et al.* 1992, Cahill 1994, Irwin *et al.* 1995). Breeding for resistance to this fungus has been investigated and shows promise for the future (McComb *et al.* 1987, McComb *et al.* 1991, Cahill *et al.* 1992, McComb *et al.* 1994, Stukely and Crane 1994). While there is much more information available on this fungus, it is out of the scope of this review.

Armillaria

Armillaria has a wide distribution (Gibson 1975, Kile *et al.* 1991, Shaw and Kile 1991). In Australia, the pathogenic species on eucalypts *A. leutobubalina* Watling and Kile, was first recorded in the 1950s (Purnell 1959, Gibson 1975, Kile 1981, Kile 1983b, Kile *et al.* 1983, Kellas *et al.* 1987). It usually occurs as an external root associate or bark surface coloniser but can attack and kill trees, especially when the vigour of the host declines in old age (Gibson 1975) Its main dispersal mechanisms are in the form of mycelia, basidiospore (although a rare event) and rhizomorphs (local infections) but can also be spread via root contacts (Marks *et al.* 1976, Kellas *et al.* 1987, Kile *et al.* 1991). Root rot associated with *Armillaria* has been widely recorded in eucalypts in Australia (Bird *et al.* 1975, Marks *et al.* 1976, Neumann and Marks 1976, Kile 1980, Kile 1981, Kile 1983a, Kile 1983b, Kile 1986, Kile and Watling 1983, Kile *et al.* 1983,Pearce *et al.* 1986, Shearer and Tippett 1988, Shearer 1995). Selective logging in old growth forests may intensify disease development and may also continue to affect the survival of any regrowth seedlings (Edgar *et al.* 1976, Kellas *et al.* 1987, Kile *et al.* 1991). *Armillaria* has the potential to cause severe damage in plantation forestry and has been shown to reduce tree growth (Edgar *et al.* 1976, Kile *et al.* 1982), but it depends heavily on the presence of an aggressive strain of the fungus (Gibson 1975, Podger *et al.* 1978, Kile 1980, Kile 1981, Benjamin and Newhook 1984, Morrison 1989, Kile *et al.* 1991). More literature is available on this

fungus and the reader is directed particularly to Gibson (1975), Marks *et al.* (1982), and Shaw and Kile (1991).

Other root diseases

Other pathogens that cause root disease include the genera *Botryosphaeria* , *Fusarium, Cylindrocladium* and *Pythium* (Marks and Kassaby 1974, Gibson 1975). *Botryoshaeria ribis* Gross and Dugg. can cause root and stem necrosis in poorly drained soils (e.g. Magnani 1964b, Gibson 1975, Swart *et al.* 1993, Smith *et al.* 1994). *Fusarium* causes seedling wilt, particularly in young seedlings (see Arya and Jain 1962, Magnani 1964a, Hepting 1971, Mahmood 1971, Sedgley 1974). Canker can also occur as a result of *Fusarium* infection (see Magnani 1964a). *Cylindrocladium* is one of the more serious fungal pathogens of eucalypts. It causes damping-off of seedlings and root rot and dieback at later stages (Gibson 1975). It is distributed widely on eucalypts around the world (see Arruda 1943, Batista 1951, Terahila and Takai 1955, Cruz and Figueredo 1961, Bakshi 1967, Figueirdo and Namekata 1967). The genus *Cylindrocladium* has been recently reviewed (see Crous and Wingfield 1994, Crous *et al.* 1994, Linde *et al.* 1994a). *C. eucalypticola* is one of the more damaging species of this genus (van der Westhuizen 1965a, van der Westhuizen 1965b, Gibson 1975). *Pythium* can also cause root and collar rot and has recently been associated with serious damage on eucalypts in South Africa (Linde *et al.* 1994b).

Other branch and stem diseases

There are a large number of stem and branch pathogens and a review of these can be found in Gibson (1975), and Heather and Griffin (1984). Included in these are *Cytospora* spp., *Botryosphaeria* spp., *Sporotrichum* spp., *Corticium* sp. and *Endothia* spp.. *Sporotrichum* is the most important of the first three species, which causes canker on eucalypt branches in Australia (Gibson 1975). *S. destructor* is particularly pathogenic to *Eucalyptus ficifolia* (Smith 1970). *Cytospora* spp, which also causes branch cankers, has host species which includes *E. ficifolia* and *E. globulus* (Azevedo 1971 cited in Gibson 1975). *C. eucalypticola* is one of the more damaging species of this genus (van der Westhuizen 1965a, van der Westhuizen 1965b, Gibson 1975). *Botryosphaeria ribis* (Tode ex Fr.) has been shown to cause collar and branch cankers (Magnani 1964b, Hepting 1971, Smith *et al.* 1994). *Corticium*, in particular *C. salmonicolor* Berk and Br., which causes pink disease, has a wide host range and is a serious pest of eucalypts overseas (Gibson 1975, Heather and Griffin 1984). *Endothia havanensis* Brener has induced serious canker, causing high mortality and the suspension of eucalypt planting in Surinam (Anon. 1969 cited in Boerboom and Maas 1970, Heather and Griffin 1984). *Eucalyptus saligna* and *E. grandis* are particularly susceptible to this disease (Anon. 1969, Boerboom and Maas

1970). In Australia, *Endothia* has been implicated with cankers on red flowering gums (Smith 1970) and most likely is associated with rot and cankers in *Eucalyptus regnans* (Old *et al.* 1986, White and Kile 1991, White and Kile 1993, White and Kile 1994). *E. grandis* is also highly susceptible to the canker pathogen *Cryptonectira cubensis*, causing large production losses in Brazil (Campinhos and Ikemori 1989). Breeding for resistance to this pathogen, particularly through the use of hybrids with *E. urophylla* has been investigated (Campinhos and Ikemori 1989). In general, pathogens causing cankers on eucalypts in plantations appear to be much more serious overseas than they are in Australia (Heather and Griffin 1984).

Butt rots

The establishment and development of rot in eucalypts is not well understood (Wilkes 1982b). The pathogens attributed to decay are even less well documented, although a few have been identified (e.g. Edwards 1982a, Wilkes 1982a, Mireku and Wilkes 1989, Wardlaw 1996). Some of the main pathogens implicated so far include *Phialophora* sp, *Armillaria*, *Geosmithia* sp., *Leptographium*, *Ceratocystis* and *Poria* sp. (Edwards 1982a, Wilkes 1982a, Wilkes 1982b, Wardlaw 1996). However, it appears that there is a succession of a wide range of fungi throughout the decay process and there is still work to be done in this area (see Edwards 1982a, Wilkes 1982a) The majority of research in the literature has focussed predominantly on the origin and patterns of decay with a view to future resistance breeding (see Wilkes 1982a, Wilkes 1985a, Wilkes 1985b, Wilkes 1985c, Wilkes 1985d, Wilkes 1986a, Wilkes 1986b, White and Kile 1991, White and Kile 1993, White and Kile 1994). Some resistance work has in fact been undertaken in a number of *Eucalyptus* species (Wilkes 1982a, Wilkes 1982b). Gibson (1975) and Heather and Griffin (1984) give a broad review on heart rot fungi.

Mechanisms of resistance

Secondary compounds

Secondary compounds in plants are largely considered to include all compounds which are non-nutritional and have no developmental affect. Although there is some debate as to the relevance of this generalisation to some 'secondary compounds' (Seigler and Price 1976, Seigler 1977), the idea has generally become accepted since it was first suggested by (Frankel 1959). Included in this group are surface waxes, phenols, glucosides, saponins, tannins, alkaloids, essential oils and organic acids (Frankel 1959, Ohmart and Edwards 1991). Eucalypts contain a large quantity of secondary compounds (Penfold and Willis 1961, Levin 1971, Li 1993). However, even though these compounds have been shown to be important in other species (e.g.

Pinus) (Becerra and Venable 1990, Michelozzi *et al.* 1990, Michelozzi *et al.* 1995), *Salix* (Kelly and Curry 1991), there is conflicting evidence on their effect in *Eucalyptus* (Ohmart and Edwards 1991).

There is some evidence that in eucalypts, cineole content is important in herbivore distribution (Edwards *et al.* 1993). Herbivory has been found to be related to cineole content in un-stressed *E. camaldulensis* (Stone and Bacon 1994) and the host preference of *Chrysophtharta bimaculata* (Chrysomelidae) (Li 1993). Similarly, a significant correlation between defoliation and the proportion of cineole in the overall terpenoid mixture was found for *Anoplognathus* spp. (Scarabidae) on several eucalypts (Edwards *et al.* 1993). However, it is always difficult to identify the exact cause of resistance. In the case of cineole, levels are not always able to be directly identified as the sole source of resistance in eucalypts because of correlations with other factors, including other oils (e.g. Stone and Bacon 1994). Tannins have been implicated to some extent in oviposition choice of *Perthida glyphora* on jarrah (*E. marginata*). Similarly, discrimination in adult beetles of *Phorocantha semipunctata* between host logs of *Eucalyptus* may be by volatile cues most likely caused by secondary compounds (Hanks *et al.* 1993). Morrow and Fox (1980) have postulated from their results on insect herbivory, that a threshold level of essential oil content rather than specific oil concentration may confer resistance. However, in some instances, no relationship between secondary compounds (tannins and phenols) and herbivory has been found at all (Fox and Macauley 1977). It appears that the issue is complicated and more work is needed in this area. Clearly, the influence of non-genetic factors on secondary compounds also needs further investigation (see Doran and Bell 1994).

Nitrogen and leaf quality

Nitrogen (N) is of major importance in insect/plant association (see Mattson 1980, Cockfield 1988). White (1969) first proposed that N may be important in host plant selection for stressed plants by increasing in particular, free amino acids which are more readily available for herbivore utilisation. These observations were based on hypotheses explaining outbreaks of psyllids (*Cardiaspina densitexta* on *E. fasciculosa)* (White 1969, White 1984). *Eucalyptus* leaves have been shown to be low in N quantity (e.g. Fox and Macauley 1977) and N may therefore be the limiting requirement for long-term herbivore survival. N has been found to be a particular requirement for the success of *Paropsis atomaria* on *E. blakelyi* (Fox and Macauley 1977, Ohmart *et al.* 1985). If N levels fell below 1.7%, insect viability was considerably reduced (Ohmart 1991). However, N level and leaf toughness may be highly correlated in this system (Ohmart *et al.* 1987) and it may be difficult to confirm N as the sole agent. Further, these results have been contradicted, debated and further

investigated by Miles *et al.* (1982). Leaf toughness has been shown to have a major role in the resistance of a number of rainforest species (Lowman and Box 1983). Leaf shape and structure can also have important effects on host preference as for example, with *Gonipterus scutellatus* on eucalypts (Richardson and Meakins 1986). Specialist herbivores showing selective feeding within plants of low nutritive quality (e.g. in holly *Ilex*, Kimmerer and Potter 1987), also outline the care with which the broader picture in plant nutrition must be viewed. Although there is limited information on the effect of leaf toughness on host selection of eucalypts, pests such as *Amorbus obscuricornis* have a clear preference for the softer, more nutritious coppicing shoots rather than the non-coppicing shoots of *E. regnans* (Steinbauer pers. comm.).

Genetic resistance

Limited work has been undertaken on the genetic basis of resistance and its inheritance in eucalypts. Although serious insect damage has been shown to occur (e.g. Carne *et al.* 1974, Elliot and Bashford 1978, Leon 1989), the possible impact on wood production has only just begun to be documented (see Floyd and Farrow 1994). Variation in resistance within species has been demonstrated for a number of herbivores including *Chrysophtharta bimaculata* (Leon 1989, Raymond 1995), *Perthida glophopa*, the jarrah leaf miner (Mazanec 1974, Mazanec 1985), *Cardiaspina* spp., *Phylactophaga froggatti* and *Mnesampela privata* (Floyd and Farrow 1994, Floyd *et al.* 1994); (see also Landsberg and Wylie 1983, Landsberg 1990a, Landsberg 1990b, Landsberg 1990c, Raymond 1995). Further, variation to insect resistance has been demonstrated at a within-tree level in *Eucalyptus* through somatic mutation (Edwards *et al.* 1990). Significant variation has also been noted for resistance to fungal pathogens, for example: *Mycosphaerella* (Wilcox 1982a, Lundquist and Purnell 1987, Carnegie *et al.* 1994) ; *Puccinia psidii* (Dianese 1984) and *Phytophthora cinnamomi* (Cahill 1994, Stukely and Crane 1994). For breeding purposes, detailed genetic information is needed to make informed selections. Heritabilities, or the proportion of variation which has an additive genetic basis at a family or individual level, are required (Becker 1985, Falconer 1986). Heritability estimates are rare for resistance traits in eucalypts. Published estimates are given in Table 1.1. The high individual narrow-sense heritability obtained for resistance to *Phytophthora cinnamomi* in jarrah (0.43) indicates that breeding for resistance would not only be possible, but would be most likely to give a reasonable gain in resistance (Stukely and Crane 1994). Similarly, the moderate heritability obtained for *Chrysoptharta bimaculata* damage (0.3 for *E. regnans* and 0.4 for *E. nitens*) indicates that breeding for resistance is feasible in this system (Raymond 1995).

Research outline

The main disadvantage of a large number of studies on natural hybrids is hybrid identification and thus verification of a genetic basis to the observed hybrid susceptibility. In general, only morphological traits were used to select intermediate 'hybrid' phenotypes. The genetic status of the hybrids was largely unknown, with the possibility that they were either F_1 or advanced generation hybrids. Further advances in studies of hybrid pest interactions will ultimately depend on comparing the responses of pests to plants of known pedigree and genotype in controlled or common environments (Fritz *et al.* 1994). This thesis directly examines the susceptibility of eucalypt hybrids to attack by fungal, insect and vertebrate taxa. The majority of work undertaken was on controlled-cross hybrids, particularly first generation (F_1) hybrids. This study has considerable advantages over most previous studies because both the genotype and phenotype of the host hybrids are known. In addition, the parental species and the corresponding hybrids are all planted in randomised experimental field trials.

Chapter 2 examines the genetic parameters and susceptibility of *Eucalyptus nitens, E. globulus, E. bicostata* and the F_1 hybrids *E. nitens x E. globulus* and *E. bicostata x E. globulus* to leaf disease caused by *Mycosphaerella*. Host preference, hybrid susceptibility and genetic parameters for disease severity are determined for this leaf disease.

Chapter 3 examines damage caused by the common brushtail possum *Trichosurus vulpecula* using two pedigreed eucalypt species trials. Qualitative host species preference and genetic parameters for possum damage are determined. The response of the possums to the pedigreed interspecific hybrids when compared with the appropriate pure parent species is also investigated.

Chapter 4 examines the host preference of chrysomelid beetles (*Chrysophtharta* spp.) and the gum leaf skeletoniser, *Uraba lugens*. Susceptibility of eucalypt hybrids and their respective pure parent species is examined. Both qualitative and quantitative estimates (chrysomelids only) of leaf area loss caused by these insects are determined. Host preference of chrysomelids in terms of relative amounts of damage caused is examined using several experimental field trials and 15 species of eucalypt.

Chapter 5 examines the species richness of a large number of insect taxa on *E. amygdalina, E. risdonii*, their F_1 and advanced generation hybrids in an experimental field trial. Results obtained are compared with results obtained previously in a natural hybrid zone between the same eucalypt species. Host preference and the genetic nature of the observed insect distribution is discussed for the different eucalypt species and

their hybrids. In addition, a number of mechanisms which may contribute to the observed responses are examined. These mechanisms are subsequently discussed with respect to the distribution of the insect taxa within the field trial and specifically in a single large segregating second generation (F_2) family.

Chapter 6 investigates the distribution of a leaf gall caused by a chalcid wasp, again on *E. amygdalina, E. risdonii* and hybrids between these two eucalypt species. Initially, the numbers of galls on pure host species and hybrid phenotypes found within a natural hybrid zone is determined. Distribution of the gall is compared with the distribution of the same gall previously determined by Whitham *et al.* (unpublished data) in the same natural hybrid zone. The repeatability of the results obtained in the natural hybrid zone is discussed. In addition, the distribution of the gall is investigated in a controlled cross field trial containing pure *E. amygdalina* and *E. risdonii*, and both F_1 and advanced generation hybrid material. The extent of genetic control responsible for the gall distribution is also discussed in light of the gall's distribution determined in this controlled-cross trial.

Chapter 7 examines the oviposition preference and larval survivorship of the eucalypt weevil *Gonipterus scutellatus* within the same pedigreed *E. amygdalina x E. risdonii* hybrid trial used in Chapters 5 and 6. General discussion follows in Chapter 8.

Table 1.1. Published heritability estimates for resistance traits in *Eucalyptus*. Family or individual narrow sense heritabilities are presented and whether they were determined from open-pollinated (op) or controlled-cross (cc) progeny trials. The coefficient of relationship, r used in the calculation of heritabilities from open-pollinated progeny, is also given (see note).

organism	host	details	h^2 type	h^2	op/cc	r	Paper
Phytophthora cinnamomi	*E. marginata*	stem inoculation	individual	0.43±0.18	op	0.40	Stukely and Crane (1994)
		field resistance	family	0.85	op	-	
		soil inoculation	family	0.78	op	-	
Chrysophtharta bimaculata	*E. regnans*	field defoliation	individual	0.13±0.05-0.41±0.07	op	0.40	Raymond (1995)
	E. nitens	field defoliation	individual	0.48±0.08	op	0.40	
Mycosphaerella	*E. globulus*	field defoliation	individual	0.32	op	0.40	Reinoso and Ades (in press)
		field severity	individual	0.31		0.40	
Mycosphaerella	*E. globulus*	field severity	individual	juvenile 0.14	cc	-	This study, Chapter 2 (Dungey *et al.* 1995)
		field severity	individual	adult 0.21	cc	-	
	E. nitens	field severity	individual	juvenile 0.12	cc	-	
		field severity	individual	adult 0.34	cc	-	
Ganoderma sculptrutum	*E. grandis* (Zimbabwe)	field infection (symptoms)	family	0-0.79	op		Masuka and Nyoka (1995)
		field infection (deaths)	family	0-0.75	op		

Note: A coefficient of relationship adjusts estimates of additive genetic variance for the relative level of inbreeding, which is usually assumed to be around 30% in eucalypts (Cameron and Cotterill 1989).

Chapter 2

Mycosphaerella spp. leaf disease: Genetic variation in damage to *Eucalyptus nitens*, *E. globulus* and their F_1 hybrid

Introduction

Eucalyptus nitens (Deane & Maiden) Maiden and *E. globulus* spp. *globulus* Labill. (hereafter referred to as *E. globulus*), are two of the most important eucalypts for plantations in temperate Australia (Tibbits 1986, Eldridge *et al.* 1994). *E. globulus* is the more widely planted of the two species (e.g. Volker and Orme 1988, Davidson 1989, Eldridge *et al.* 1994). It occurs naturally in coastal regions of Tasmania, the Bass Strait Islands and southern Victoria (Kirkpatrick 1975, Jordan *et al.* 1993). *E. nitens* naturally occurs from central Victoria to northern NSW in a number of widely disjunct populations (Pederick 1979, Neish *et al.* 1995). *E. nitens* is becoming increasingly important as a plantation species, particularly in Tasmania, largely because of its high level of frost resistance compared with *E. globulus* (Tibbits and Reid 1987a, Tibbits and Reid 1987b, Volker *et al.* 1994).

E. globulus and *E. nitens* are both susceptible to *Mycosphaerella* leaf disease which causes leaf necrosis and defoliation (Park and Keane 1982a, Wilcox 1982a, Purnell and Lundquist 1986, Lundquist and Purnell 1987, Carnegie and Keane 1994, Carnegie *et al.* 1994). Leaf damage can be severe and highly detrimental to growth (Park and Keane 1982a, Lundquist 1985, Lundquist and Purnell 1987, Carnegie *et al.* 1994). Two species of *Mycosphaerella* have been recorded as causing significant disease on *E. globulus*: *M. molleriana* (Thüm.) Lindau (Syn. *M. nubilosa* (Cooke) Hansf., Crous *et al.* 1991) which occurs on the juvenile foliage, and *M. cryptica.* (Cooke) Hansf. which occurs on both juvenile and adult foliage (Park and Keane 1982b, Carnegie *et al.* 1994). Disease of both the juvenile and adult foliage of *E. nitens* in Australia, is believed to be caused by *M. cryptica* (Carnegie *et al.* 1994).

In eucalypts, studies of genetic variation in resistance to fungal pathogens have concentrated on differences between provenances (Marks and Idczak 1977, Dianese 1984). Provenance variation in resistance to *Mycosphaerella* leaf disease has been reported in *E. globulus* (Carnegie *et al.* 1994), *E. nitens* (Purnell and Lundquist 1986), *E. regnans* (Wilcox 1982a, Dick and Gadgil 1983) and *E. delegatensis* (Dick and Gadgil 1983). Significant differences between families within a provenance have

also been reported in *E. globulus* (Reinoso and Ades in press) and *E. regnans* (Wilcox 1982a) but there have been few detailed studies of the genetic control of resistance to *Mycosphaerella* leaf disease. Reinoso and Ades (in press) have reported individual narrow-sense heritabilities of h^2 =0.31 and 0.32, for *Mycosphaerella* leaf disease derived from open-pollinated progeny from a single provenance of *E. globulus*. However, due to unpredictable, and possibly differential inbreeding in open-pollinated progeny, the accuracy of such estimates have been questioned (Hardner and Potts 1995, Potts *et al.* 1995, Hodge *et al.* 1996). Accurate estimates of genetic parameters often require fully pedigreed controlled crosses (Potts *et al.* 1995, Hodge *et al.* 1996). They also provide an indication of the importance of non-additive genetic variation in the genetic control of a trait.

The aim of this study is to examine the inheritance of severity of *Mycosphaerella* leaf disease occurring in intra- and interspecific crosses of *E. globulus* and *E. nitens*. In addition, we compare the accuracy of genetic parameters and breeding values derived from open-pollinated and fully pedigreed progenies.

Materials and Methods

Field trial

Disease was assessed in a field trial containing progeny from an incomplete 8 x 26 *E. globulus* factorial, an incomplete 10 x 10 *E. nitens* half-diallel, an incomplete 6 x 14 *E. nitens x E. globulus* F_1 hybrid factorial and open-pollinated progeny (See Table 2.1 and (Volker *et al.* 1994). The *E. globulus* parents were from three provenances: Taranna (T), King Island (K), and South Flinders Island (SF). The factorial included both intra- (T, K or SF) and inter-provenance (TxK, KxT, SFxT or SFxK) crosses (see Table 2.1, Volker and Orme 1988, Jones *et al.* 1993, Volker *et al.* 1994, Potts and Jordan 1994b). All *E. globulus* males were growing in native stands at Taranna or King Island. All *E. globulus* females were from open-pollinated progeny growing in a seedling seed orchard in north-west Tasmania and originated from native stands at either Taranna, south Flinders Island or King Island (Volker *et al.* 1990, Volker *et al.* 1994). Due to the lack of replication, progeny of the single female from the SF provenance were excluded from all analyses (see Table 2.1). All the *E. nitens* parents were from the Toorongo provenance (Pederick 1979) and were growing in seedling seed orchards or in plantations in north-west Tasmania. Open-pollinated (OP) progeny of all the males (native stand OP) and females (seed orchard OP) were also included. The *E. nitens x E. globulus* F_1 hybrids included *E. globulus* males from both King Island and Taranna provenances. All males used in the *E. nitens x E. globulus* hybrid factorial were also used in the *E. globulus* factorial and all females

were used in the *E. nitens* half-diallel. In addition to the control-cross and open-pollinated material, some unpedigreed *E. globulus* ssp. *bicostata* (Maiden, Blakely and J. Simm.) Kirkpatr. (hereafter called *E. bicostata*), and *E. bicostata x E. globulus* F_1 hybrids were included in the trial (four and six families respectively) but there were insufficient progeny to estimate genetic parameters for these cross types.

The number of families and individuals in each cross type are given in Table 2.2 and trial details are given in Table 2.3 and Volker *et al.* (1994). The trial was established by CSIRO Division of Forestry and North Forest Products, at West Ridgley in north-west Tasmania. The trial contained approximately 6000 trees and was based on an alpha lattice design (Patterson and Williams 1976). Each of the four replicates of 1500 trees was comprised of 15 incomplete blocks, with 20 line plots of 5 trees per incomplete block (see also Hodge *et al.* 1996).

Disease assessment

Disease severity on leaves still retained on the trees was assessed separately for both the juvenile and adult portions of the crown following Reinoso and Ades (in press), using percentage damage diagrams adapted from Carnegie *et al.* (1994) and Lundquist and Purnell (1987). The severity classes used were 0, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, and 60% leaf area killed. Both *M. cryptica* and *M. molleriana* were present but lesions were difficult to distinguish in the field and, following Reinoso and Ades (in press), only total disease severity was assessed. Trees were assessed in mid March 1993, when approximately 3 years old. There was no significant disease damage in the previous year and that present at the date of assessment had become apparent in the 1992-93 summer season. *Mycosphaerella* spp. cause both leaf necrosis and later, defoliation. It appears that these responses are independent and measure different components of susceptibility (Reinoso and Ades in press). At the time of assessment, minimal defoliation due to *Mycosphaerella* disease had occurred and our results only refer to the first component of susceptibility, termed 'disease severity' by Reinoso and Ades (in press).

The relationship between percentage disease severity and tree size immediately prior to the onset of infection (height, DBH and volume 1992) and subsequent growth (increments from 1992-1993) were examined. The correlation between percentage disease severity and size of the juvenile canopy was also investigated. Measurements were taken in August 1992 (2 year) and September 1993 (3 year), for diameter at breast height (DBH) and height to phase change (the transition between juvenile and adult foliage; Potts and Jordan 1994a). Tree height was measured only in the second and third years (August 1992 and September 1993). Individual conic volume was calculated following Potts and Jordan (1994a).

Data analysis

All runts and plants with highly abnormal phenotypes were excluded from the analyses (8.2 percent of the total number of individuals). All data were then log transformed to obtain approximate normality of the residuals. The *E. globulus* factorial, *E. nitens* half-diallel and *E. nitens x E. globulus* F_1 factorial were analysed separately. Genetic parameters for disease and genetic correlations with growth characters were calculated following Volker *et al.* (1994) using REML VCE (Groeneveld 1995). The individual tree model used was [1]:

$$[1] \quad y = X_1c + X_2r + Z_1a + Z_2s + Z_3b + Z_4p + e$$

where y is an nx1 vector of individual *Mycosphaerella* spp. damage observations, *c* is a vector of fixed crosstype effects, *r* is a vector of fixed replicate effects, *a* is a vector of additive genetic effects (i.e. breeding values of individuals and parents), *s* is a qx1 vector of random genetic effects common to each full-sib family (i.e. specific combining ability), *b* is a bx1 vector of random effects common to each incomplete block (within each replicate), *p* is a px1 vector of random effects common to each plot (i.e. plot effect), and *e* is an nx1 vector of residuals, expected to include mainly the remaining three quarters of the dominance variance and environmental effects. X_1, X_2, Z_1, Z_2, Z_3, and Z_4 are known incidence matrices relating observations in y to effects in *c, r, a, s, b* and *p* respectively. Where specific cross types were examined (e.g. *E. nitens,* or within *E. globulus* Taranna or King Island provenances only), the cross effect was omitted from the model. Open-pollinated progeny were analysed using model [1], excluding the full-sib family term (Z_2s).

Estimates of additive (σ^2_a), SCA (σ^2_s), incomplete block (σ^2_b), plot (σ^2_p) and error (σ^2_e) variance components were used to calculate individual narrow-sense heritabilities (h^2) and the proportion of dominance variance (d^2) as follows:

$$h^2 = \sigma^2_a/(\sigma^2_a + \sigma^2_s + \sigma^2_p + \sigma^2_e)$$

$$d^2 = \sigma^2_d/(\sigma^2_a + \sigma^2_s + \sigma^2_p + \sigma^2_e)$$

where $\sigma^2_d = 4 * \sigma^2_s$ (Falconer 1986). Estimates of heritability for the open-pollinated populations were calculated as given above but excluding σ^2_s. Such estimates assume that the open-pollinated families are half-sibs (r=0.25). However, this estimate was adjusted (h^2_{op}) to account for selfing within open-pollinated families of *Eucalyptus* by multiplying the h^2 estimates by 0.625. This adjustment follows Griffin and Cotterill (1988) and Volker *et al.* (1990) and assumes an average outcrossing rate of 70% and a genetic correlation (r) amongst open-pollinated sibs of 0.4. Standard errors were estimated following Becker (1985).

Cross type least squares means were determined using the MIXED procedure in SAS (1992) using model [1]. Contrasts were undertaken to test differences between cross types and whether hybrids were significantly different from the expected mid-parent value using PEST (Groeneveld *et al.* 1992), which performs an F test based on estimates of the error variance (Kennedy 1989).

Best linear unbiased predictions (BLUPs) of parental breeding values (BVs) were calculated with PEST (Groeneveld *et al.* 1992), using variance component estimates from REML VCE. BVs were calculated separately for the *E. globulus*, *E. nitens* and F_1 hybrid controlled cross populations and the *E. globulus* and *E. nitens* open-pollinated populations. This allowed direct comparison of parental BVs estimated in pure species and hybrid combination as well as under open-pollination. The model used in these analyses was the same as [1], but the cross type effect was excluded for the *E. globulus* parents to enable parental breeding values to be estimated across provenances. Parental breeding values were similarly estimated for the *E. globulus* males using the open-pollinated and hybrid populations and these estimates were compared with those determined from controlled intraspecific crossing.

Results

Cross type effects

There was significant provenance variation within *E. globulus,* with Taranna (T) having a significantly higher average disease severity than the King Island (K) provenance on both juvenile (P<0.01) and adult foliage (P<0.001) (see Table 2.4 and Figure 2.1). The inter-provenance crosses between Taranna and King Island parents (TxK and KxT) exhibited intermediate severity and were not significantly different from the mid-provenance value in either juvenile or adult foliage (Table 2.4). Disease severity on juvenile foliage of the Toorongo provenance of *E. nitens* (N) was not significantly different from that on *E. globulus* from Taranna, but was significantly (P<0.05) higher than on the King Island provenance. On adult foliage, disease

severity on *E. nitens* was significantly lower than for any other cross type (P<0.05). Disease severity was significantly lower on the juvenile foliage of *E. bicostata* when compared with *E. globulus* (P<0.001). However, on adult foliage severity was not significantly different between *E. bicostata* and *E. globulus* (P= 0.167).

In general, the disease severity on juvenile foliage of the *E. nitens* x *E. globulus* F_1 hybrids was significantly (P<0.05) greater than for either *E. nitens* or *E. globulus* (see Figure 2.1, Table 2.4), regardless of whether the provenances of *E. globulus* were pooled or not (except N vs NxK n.s.). The disease severity on the adult foliage of the F_1 hybrids was significantly greater (P<0.001) than on *E. nitens*. However, the NxK hybrids had similar severity to the King Island provenance, while the NxT hybrids were intermediate, with significantly less disease than the Taranna provenance. The *E. bicostata* x *E. globulus* hybrids (BxG) had the higher disease severity on adult foliage than any other cross type in the trial, including both parent species (P<0.001). On juvenile foliage, the *E. globulus x E. bicostata* hybrids had significantly higher severities than *E. bicostata*, but were not significantly different from the pooled *E. globulus* provenances (King Island and Taranna). In contrast to the *E. globulus* inter-provenance crosses, the interspecific crosses of *E. globulus* with *E. nitens* or *E. bicostata*, exhibited disease severities significantly (P<0.01) greater than the predicted mid-species values for both adult and juvenile foliage types (Table 2.4).

Genetic Parameters

Controlled-cross estimates of individual narrow-sense heritability (h^2) of the percentage of leaf area damaged ranged from 0.115 to 0.343 for *E. globulus* and 0.004 to 0.208 for *E. nitens* (Table 2.5). Heritability estimates for adult foliage were always greater than those for juvenile foliage, despite the disease severity being greater on juvenile foliage (see Table 2.5, Figure 2.1). There was a tendency for estimates of h^2 in both adult and juvenile foliage to be lower for *E. nitens* than for *E. globulus*. Furthermore, when the provenances of *E. globulus* were separated, the h^2 for the King Island population (juvenile 0.115, adult 0.343) were comparable with those obtained for the Taranna population (juvenile 0.120, adult 0.250). The proportion of dominance variation (d^2) was low (Table 2.5), particularly for the *E. nitens* population, and in most cases was less than half of the h^2 estimate. The h^2 estimate for damage on juvenile foliage in the King Island population was the lowest of the *E. globulus* estimates and this was the only case where the d^2 estimate exceeded h^2. These results suggest that in most cases, there is little non-additive genetic variation for susceptibility in these eucalypt populations and most of the genetic variation is due to additive effects. Estimates of d^2 for the F_1 hybrid population were comparable with those obtained for the pure species. In contrast, the

h^2 estimate for juvenile foliage was nearly double that found in any of the pure species populations. Estimates of h^2 for disease severity on the adult foliage of the F_1 hybrids were consistent with the pure species estimates. Estimates of h^2 based on the open-pollinated progeny (h^2_{op}) collected from the *E. globulus* males were comparable to those obtained from controlled crossing (Table 2.5). In both *E. globulus* and *E. nitens* the h^2_{op} estimates for juvenile foliage were larger than the h^2 estimates, whereas those obtained from adult foliage were smaller. However, the standard errors of the h^2 estimates would suggest that these differences were not significant.

Genetic correlations between disease severity scores and growth traits are given in Table 2.6. The percentage severity in the adult canopy was highly genetically correlated with severity in the juvenile canopy in all populations (0.52 to 0.74, see Table 2.6). In the *E. globulus* population, neither the adult nor juvenile severity was genetically correlated to tree size at the presumed time of infection as genetic correlations between damage and height or DBH in 1992, were low (Table 2.6). Growth, measured as the increment in DBH, height or volume over the summer season in which the infection occurred, was consistently positively genetically correlated with severity in the juvenile canopy. This correlation indicated that at the genetic level, increased *Mycosphaerella* damage was associated with an increase in subsequent growth. While contrary to what may be expected, this appears to be due to the delayed phase change in *E. globulus* being genetically associated with both increased growth (r=0.61 between HTPC and sectional area at age 4, P. Volker, unpubl. data) and increased damage to the juvenile canopy (r=0.54, Table 2.6). In contrast, correlations of incomplete block effects between damage to the juvenile foliage and subsequent growth traits, which reflect an environmental correlation, were consistently negative (-0.26 to -0.38) as would be expected if disease was having a direct deleterious effect on growth.

In the *E. nitens* population, there was a general negative correlation between disease on both the adult and juvenile foliage and tree size at the presumed time of infection. The genetic correlations between disease severity on the juvenile foliage and subsequent growth were inconsistent, but negative for adult foliage (Table 2.6). Within the *E. nitens* x *E. globulus* F_1 hybrid population, severity of disease on both the juvenile and adult foliage were positively correlated with all the growth traits measured. Tree size at the time of infection (DBH and height at 2 years), appears to have had a profound effect on disease severity, with faster growing hybrids having higher scores for both juvenile and adult foliage (Table 2.6). A larger juvenile canopy (larger HTPC) was strongly genetically correlated with severity on both the juvenile and adult foliage. This contrasts with both *E. globulus* and *E. nitens*, where high

severity in the adult canopy was generally associated with a smaller juvenile canopy (HTPC).

Correlations of parental breeding values

Parental breeding values (BVs) for disease severity in either juvenile or adult foliage were similar for *E. globulus* (Pearson's correlation coefficient, r = 0.69, see Table 2.7), consistent with the high genetic correlation given in Table 2.6. Likewise, parental BVs estimated from controlled-cross progeny and open-pollinated progeny were highly correlated in both *E. globulus* and *E. nitens* (Pearson's correlations of 0.73-0.75 and 0.50-0.73 respectively, Table 2.7). The distinction between *E. globulus* parents from the King Island provenance (which generally had lower BVs, corresponding with lower disease severity), and parents from Taranna for these correlations can be seen in Figure 2.2. The correlations between parental BVs calculated from controlled-crossed progeny were poorly correlated with those calculated from parents in hybrid combination for either species (Table 2.7). Correlations between parental BVs obtained for *E. nitens* in any combination were consistently lower than those obtained for *E. globulus*, perhaps due to the low number of parents (11) and the single provenance origin and therefore limited variability of the *E. nitens* parents (Toorongo).

Discussion

There was a significant difference in the severity of damage from *Mycosphaerella* spp. lesions between the two provenances of *E. globulus,* King Island and Taranna. King Island was less damaged and this is consistent with there having been more intense natural selection for resistance to *Mycosphaerella* disease in that area. Warm, wet weather is conducive to *Mycosphaerella* outbreaks (Park 1988a, Carnegie *et al.* 1994). The wetter maritime climate on King Island (1015mm annual rainfall), would suggest a general climatic regime more favourable to *Mycosphaerella* spp. than that of Taranna (892mm annual rainfall, Figure 2.3). The lower susceptibility of *E. globulus* provenances from areas with high summer rainfall was noted by (Carnegie *et al.* 1994). *E. bicostata* (Mansfield provenance), has previously been found to have very high disease severity, when compared with *E. globulus* (Carnegie *et al.* 1994). The *E. bicostata* in the trial studied here had the lowest disease severity on juvenile foliage and on adult foliage, had similar damage levels to *E. globulus.* The relative resistance of *E. bicostata* in the West Ridgley trial appears to conflict with the severe disease on *E. bicostata* noted by (Carnegie *et al.* 1994). However, the unknown origin of material in this experiment and the low number of families means that the two results can not really be compared. *E. bicostata* is widespread and occurs in areas with both

winter and summer rainfall regimes. Provenance variation in this species has not been comprehensively tested in Australia so it is not possible to generalise about its susceptibility relative to *E. globulus*.

Disease severity was higher on interspecific hybrids than on parental populations, both on the juvenile (*E. nitens x E. globulus*) and adult (*E. bicostata x E. globulus*) foliage. Interspecific hybrids performed at least as badly as the worst performing parent in terms of the levels of damage and were consistently more damaged than the predicted mid-parent value. In contrast, the disease severity on the inter-provenance hybrids of *E. globulus* was intermediate to those on both the Taranna and King Island provenances and was not significantly different from the mid-parent values. The tendency for greater susceptibility of interspecific hybrids is consistent with other observations suggesting that interspecific hybrids may be more susceptible to pests (Whitham 1989, Fritz *et al.* 1994, Strauss 1994).

Within populations, low to moderate heritabilities (h^2) were obtained for both *E. globulus* and *E. nitens*. Heritabilities obtained for juvenile foliage were usually lower than adult foliage, despite mean severity levels being greater on juvenile foliage. The *E. nitens x E. globulus* hybrids had overall, the most severe disease (juvenile foliage 8%) and the highest heritability (0.40). Our heritability estimates for juvenile foliage were generally lower than the 0.32 reported for open-pollinated progenies by (Reinoso and Ades in press). The severity of *Mycosphaerella* leaf disease was greater at the sites used by (Reinoso and Ades in press), with damage up to 16.4%. The expression of genetic variation in disease resistance may also be dependent on the level of infection (White and Hodge 1989), and it is possible that larger heritabilities would have been obtained with greater damage on the trees used in the present study. It is unlikely that the heritability estimates of (Reinoso and Ades in press) are inflated due to the use of open-pollinated progeny, since the present study has shown such estimates to be comparable with those obtained from controlled crossing. In contrast, open-pollinated versus controlled-cross estimates have been shown to be inflated for growth traits (Hodge *et al.* 1996).

The high correlations obtained between parental breeding values estimated from open-pollination and controlled crossing of *E. globulus* clearly indicates that determining the best or worst parents for *Mycosphaerella* damage from open-pollinated progeny would be an acceptable strategy in this instance. This is an important finding as breeding values calculated from open-pollinated progeny and controlled-cross progeny for growth traits are not always well correlated (see Hodge *et al.* 1996) and, as controlled-crossing is expensive, this would significantly reduce the cost of any breeding program. Comparable correlations calculated for the *E. nitens* parents were

also high, but were not significant for adult foliage. This is most likely due to the relatively low number of parents involved in the half-diallel mating design used and the fact that all parents were from the same provenance (Toorongo). In contrast, the correlation between parental breeding values estimated in hybrid and pure species combination were always low and not significant. Hence, selecting the best parents from pure species populations for hybrid combination would not necessarily produce the best performing hybrid progeny.

Previous reports have suggested that a reduction in growth rate of *E. nitens* will not occur unless defoliation of the juvenile crown by *Mycosphaerella* exceeded 25% (Lundquist and Purnell 1987). While the percentage damage scores used here differ (see Lundquist and Purnell 1987, Reinoso and Ades in press), our results support this idea. In our trial, growth may well have been reduced by *Mycosphaerella* leaf disease, but genetic correlations between growth and disease severity of the juvenile foliage were predominantly positive, that is, the greater the growth rate the greater the disease severity score. Previous work has suggested that in *E. nitens* at least, trees with juvenile-persistent foliage are the fastest growing (Beadle *et al.* 1989). Trees with juvenile-persistent foliage also appear to be more susceptible to *Mycosphaerella* disease, possibly due to greater opportunity for disease increase due to auto-infection within the larger crown. Genetic correlations with the height to phase change provided support for this interpretation, as delayed transition to adult foliage (i.e. a larger juvenile canopy) was consistently positively correlated with disease severity. However, in the present case, disease does not appear to have been sufficiently severe to counteract the positive effect of the larger juvenile canopy on growth. Hence it seems that trees with a larger canopy or leaf area index have a greater likelihood of experiencing severe *Mycosphaerella* disease than trees with a small canopy. Reinoso and Ades (in press) have suggested that one means of increasing resistance to *Mycosphaerella* damage would be to select for early transition to adult foliage. This would reduce the period in which the plantation is highly susceptible to the disease and result in increased growth rate on sites favourable to epidemics. However, this may have an overall negative impact on growth by reducing the more productive juvenile canopy (Beadle *et al.* 1989). The fact that most tree breeders select for growth in the absence of disease (e.g. Borralho *et al.* 1992, Jarvis *et al.* 1995) means that breeders may be indirectly selecting for more susceptible trees which may have important economic implications when the stock is planted where severe disease is likely.

Acknowledgements

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Table 2.1. The number of individuals in each controlled-cross and open-pollinated family for *E. globulus* intra- and inter- provenance crosses, *E. nitens x E. globulus* F_1 hybrids and *E. nitens* half-diallel in the hybrid trial at West Ridgley in 1992. Parents from the Taranna (T), King Island (K) and South Flinders Island (SF) provenances of *E. globulus* and from the Toorongo provenance of *E. nitens* (TO) were included. GSOP is *E. globulus* open-pollinated progeny from a seedling seed orchard, GOP is *E. globulus* open-pollinated progeny from parents in natural stands and NOP is open-pollinated progeny from an *E. nitens* seedling seed orchard (see Volker *et al.* 1994).

E. globulus factorial

Female parents	Male parents: T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	K17	K18	K19	K20	K21	K22	K23	K24	K25	K26	GSOP
K-a	20	19	19	18	20	18	20	16	20	19	20	20	20		18	20	20	20	19	19	20	20	20	20	20	20	17
K-b	20	20	20	19		18	10	16	14	19	20	17	19	20	17	32	14	20		20	19	17	20	13	20	20	19
K-c	20	13				16	12	13	18	12	18	11	18	20	17	14	19	20	20	19		20	19	18	20	20	14
SF-d	20	19		20	16	17	20	19	20	20	15	11	20	14	9	19	20	20	20	20	20	20	20	20	20	20	20
T-e	18	19	10			15	20	13	17		18	18		15	18	20		18		16							13
T-f	15	11	15	15	9	10	15	15	14	7	12	13	14	17	4	14	17							15		13	16
T-g	18	20	20	15	19	18	20	20	20	20	19	19	20	18	18	20	20	20	20	20	20		20	20	20		19
T-h	17	16	12			15		13		16	12	16	12	15		13		19	17		19				16		11
GOP	15	18		19	20	16	10	23		19	9	16	15	14	17	16	19	18	20	20	18	20	20	20	20	19	

F_1 hybrid factorial

TO-k	14				11																						
TO-l				19			20			12	10	9			20					7						15
TO-m				20		9					5	12	14		17												9
TO-n	12			20		17				14	11														17		8
TO-o				17		16					10									10							9
TO-p						15				19		7								12							4

E. nitens half-diallel

Female parents	male parents: TO-k	TO-l	TO-m	TO-n	TO-o	TO-p	TO-q	TO-r	NSOP
TO-i	20	13	11	20	20	17	17	20	20
TO-j	20	18	19	20	20	18	20		20
TO-k		20		20	19		17	19	20
TO-l			20		20	18			19
TO-m				20	20		14	17	20
TO-n		20			20	20	20	17	20
TO-o						14	20		20
TO-p								20	20

Table 2.2. The number of families and individuals of each cross type in the trial at West Ridgley. (OP = open-pollinated). Provenances are in brackets following species names. *E. globulus* pooled contains all the *E. globulus* inter- and intra-provenance crosses in the trial.

Cross type	number of families	number of individuals
E. globulus pooled	168	3486
E. globulus (King Island)	28	579
E. globulus (Taranna)	55	1096
E. globulus OP	25	516
E. globulus seed orchard OP	8	155
E. nitens (Toorongo)	36	714
E. nitens seed orchard OP	9	194
E. nitens x E. globulus F_1 hybrid	43	665

Table 2.3. Establishment, climatic and measurement details for the *E. nitens x E. globulus* hybrid trial near West Ridgley, north west Tasmania.

Details	**West Ridgley field trial**
Latitude	41° 09'
Longitude	145° 46'
Altitude	185m
Establishment date	July 1990
Average annual rainfall	1200mm
Average maximum temperature	15.3°C
Average minimum temperature	7.3°C
Warmest month	February (average daily max. 22°, min. 13.°C)
Coldest month	July (average daily max. 10.5°, min. 4°C)
Geology	Tertiary basalt
Soil	Kraznozem derived from tertiary basalt. Deep, well structured, fertile and well drained.
No. replicates	4
No. incomplete blocks/replicate	15
No. plots/incomplete block	20
No. trees/ plot	5
Spacing	3x4m

Table 2.4. Contrasts between cross types for damage to *Mycosphaerella* spp. on juvenile and adult foliage of all the cross types within the hybrid trial. All contrasts were determined using PEST (Groeneveld *et al.* 1992). In the model used, cross and replicate were treated as fixed effects, incomplete block, plot and family were treated as random effects. Differences between the means of percentage disease severity are given as cross 1 minus cross 2.

contrast	juvenile		adult	
(cross1 vs cross2)	P value	difference (means)	P value	difference (means)
pooled glob vs N	0.2524	-0.557	0.0000	1.448
pooled glob vs B	0.0004	1.614	0.1671	0.604
pooled glob vs BxG	0.0904	-0.828	0.0000	-1.406
pooled glob vs F_1 (NxT and TxN)	0.0443	-2.367	0.3503	0.215
T vs K	0.0062	1.415	0.0000	1.963
T vs (TxK and KxT)	0.0048	0.889	0.0000	1.237
K vs (TxK and KxT)	0.0881	-0.526	0.0001	-0.726
Mid T&K vs pooled (TxK & KxT)	0.4016	0.889	0.1258	0.256
N vs T	0.5562	-0.211	0.0000	-2.515
N vs K	0.0341	1.204	0.0190	-0.552
NxT vs N	0.0000	2.684	0.0000	1.566
NxK vs N	0.0856	0.936	0.0000	0.900
NxT vs T	0.0007	2.473	0.0018	-0.949
NxK vs K	0.0004	2.139	0.2302	0.348
N vs (NxT and NxK)	0.0003	-1.810	0.0000	-1.233
Mid N&K vs NxK	0.0014	-1.537	0.0016	-0.624
Mid N&T vs NxT	0.0000	-2.578	0.0060	-0.308
B vs BxG	0.0000	-2.442	0.0000	-2.010
Mid (B&pooled glob) vs BxG	0.0005	-1.635	0.0000	-1.708

NOTE: T= *E. globulus* Taranna provenance, K= *E. globulus,* King Island provenance, TxK = *E. globulus* Taranna x King Island inter-provenance cross, KxT = *E. globulus* King Island x Taranna inter-provenance cross, N = *E. nitens*, B = *E. bicostata*, BXG = *E. bicostata x E. globulus* F_1 hybrid, pooled glob = T, K, TxK, KxT pooled.

Table 2.5. Components of variance, proportion of dominance variation (d^2) and individual narrow-sense heritability estimates (h^2) and their approximate standard errors (se) for *Mycosphaerella* spp. resistance in *Eucalyptus globulus*, *E. nitens* and their F_1 hybrid.

	components of variance						
cross type	additive	incomplete block	plot	family (SCA)	residual	d^2	h^2 ±se
E. globulus **(pooled)**							
juvenile	0.008	0.008	0.011	0.001	0.047	0.060	**0.119** ±0.024
adult	0.017	0.006	0.010	0.002	0.046	0.107	**0.227** ±0.032
E. globulus **(King Island)**							
juvenile	0.006	0.009	0.003	0.003	0.040	0.231	**0.115** ±0.058
adult	0.024	0.001	0.008	0.001	0.037	0.057	**0.343** ±0.101
E. globulus **(Taranna)**							
juvenile	0.009	0.010	0.012	0.000	0.054	0.000	**0.120** ±0.042
adult	0.020	0.012	0.011	0.003	0.046	0.150	**0.250** ±0.060
E. globulus **open-pollinated**							
juvenile	0.019	0.003	0.013	0.001	0.034	N/E	**0.177** ±0.072
adult	0.020	0.004	0.011	0.004	0.030	N/E	**0.192** ±0.076
Eucalyptus nitens							
juvenile	0.005	0.022	0.004	0.000	0.032	0.000	**0.122** ±0.052
adult	0.026	0.006	0.023	0.002	0.083	0.059	**0.194** ±0.065
E. nitens **open-pollinated**							
juvenile	0.018	0.016	0.004	0.001	0.031	N/E	**0.208**±0.058
adult	0.008	0.011	0.000	0.000	0.117	N/E	**0.004**±0.053
E. globulus x E. nitens							
juvenile	0.044	0.022	0.008	0.001	0.034	0.046	**0.506** ±0.010
adult	0.011	0.004	0.012	0.002	0.037	0.129	**0.177** ±0.057

NOTE: N/E = not estimable. The phenotypic variance used to calculate $d^2$and h^2 did not include the variance component due to incomplete blocks.

Table 2.6. Genetic correlations between disease severity on juvenile and adult foliage and growth traits prior to infection and incremental growth after initial infection, for *E. nitens, E. globulus,* and *E. nitens x E. globulus* hybrids. *E. globulus* pooled estimates included both the King Island (K) and Taranna (T) provenances as well as the inter-provenance crosses (both KxT and TxK). DBH = diameter at breast height. HTPC was the height to vegetative phase change (i.e. the height at which juvenile foliage changed to adult foliage).

	***E. globulus* (pooled)**		***E. nitens***		***E. nitens x E. globulus***	
	juvenile	adult	juvenile	adult	juvenile	adult
damage on adult	0.570	1.000	0.521	1.000	0.735	1.000
height at 2 years	-0.122	0.302	-0.342	-0.307	0.539	0.386
DBH at 2 years	0.195	0.045	-0.119	-1.000	0.777	0.610
growth (height 2-3 years)	0.540	0.048	0.368	-0.816	0.616	0.524
growth (DBH 2-3 years)	0.702	0.256	-0.135	-0.754	0.703	0.560
growth (volume 2-3 years)	0.469	0.105	0.012	-0.870	0.738	0.548
HTPC (3 years)	0.538	-0.311	0.617	-0.595	0.717	0.501

Table 2.7. Pearson's correlation coefficients (± s.e.) between controlled cross estimates of breeding values and those estimated for the same parents in open-pollination and in hybrid combination for *E. nitens* and *E. globulus*. Standard errors and significance levels were calculated according to Zar (1984).

		controlled-cross	significance
E. nitens			
juvenile	hybrid	**0.26**± 0.48	0.50<P<0.20
	open-pollinated	**0.73**± 0.26	**0.02<P<0.01**
adult	hybrid	**0.31**± 0. 39	0.50<P<0.20
	open-pollinated	**0.50**± 0.33	0.10<P<0.05
juvenile vs. adult	controlled-cross	**0.40**± 0.31	0.20<P<0.10
E. globulus			
juvenile	hybrid	**0.24**± 0.27	0.50<P<0.20
	open-pollinated	**0.75**± 0.14	**P<0.001**
adult	hybrid	**0.40**± 0.25	0.20<P<0.10
	open-pollinated	**0.73**± 0.14	**P<0.001**
juvenile vs adult	controlled-cross	**0.69**± 0.15	**P<0.001**

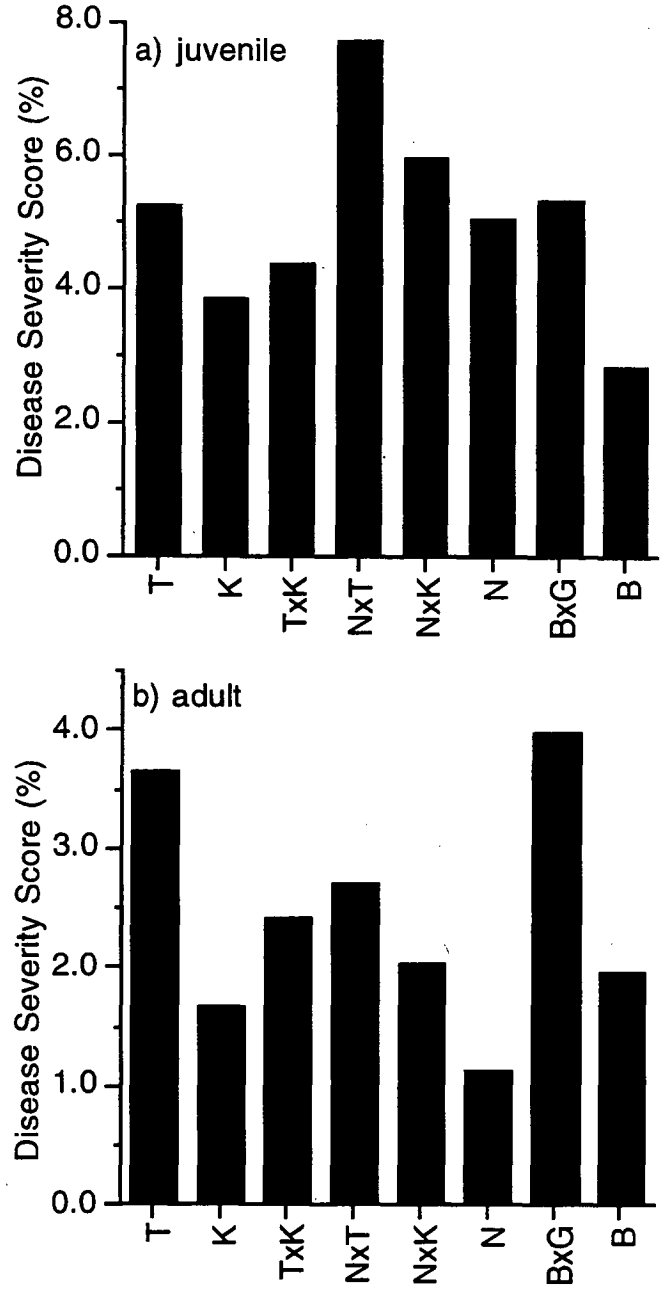


Figure 2.1. Least squares mean percentage *Mycosphaerella* spp. damage on juvenile (a) and adult (b) foliage. All cross type means were estimated using the MIXED procedure in SAS (1992). Cross types included the Taranna (T) and King Island (K) provenances of *E. globulus*, inter-provenance crosses within *E. globulus* TxK, (incorporating both TxK and KxT crosses), *E. nitens* (N), interspecific crosses between *E. nitens* and *E. globulus* (NxT and NxK), *E. bicostata* (B), and interspecific crosses between *E. bicostata* and *E. globulus* (BxG). Specific contrasts and their significance are given in Table 2.4.

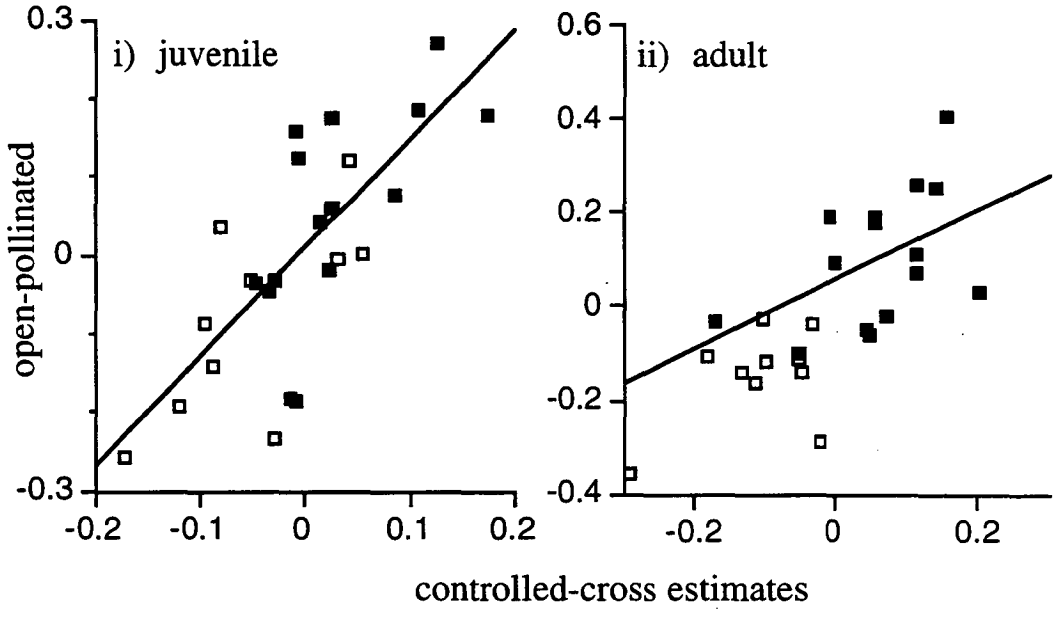


Figure 2.2. The correlation of breeding values of *E. globulus* parents in open-pollinated and controlled-cross combination for, i) juvenile and ii) adult foliage. Breeding value estimates included differences between the Taranna (■) and King Island (□) provenances of *E. globulus*. Corresponding Pearson's correlation coefficients are given in Table 2.7.

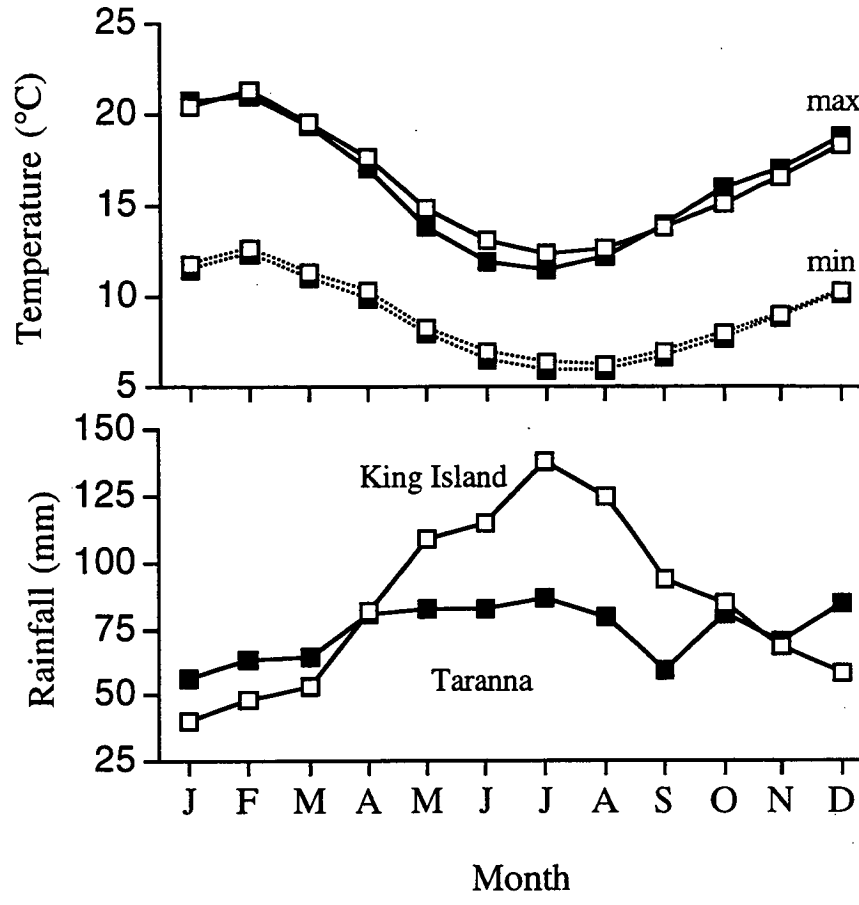


Figure 2.3. Average monthly minimum (···) and maximum (——) temperatures (°C) and rainfall (mm) for the Taranna (■) and King Island (□) provenances of *E. globulus*. All data was obtained from ESOCLIM climatic surfaces (H. A. Nix, J. P. Busley, M. F. Hutchinson and J. P. McMahon; Hutchinson 1991).

Chapter 3

Genetic variation in possum damage to inter- and intra-specific crosses of *Eucalyptus*

Introduction

The common brushtail possum (*Trichosurus vulpecula*) is widespread throughout southern and eastern Australia (Statham 1983, Kerle 1984) and has been known to cause browsing damage to eucalypts in Tasmania, (e.g. *Eucalyptus regnans* F. Muell. Mollison 1960, Cremer 1969). While there are no documented cases of serious damage in natural Australian forests, brushtails are a serious pest of forests in New Zealand (see Fitzgerald 1976, Fitzgerald 1978, Fitzgerald and Wardle 1979, Statham 1983, Fitzgerald 1984) and possum damage is becoming more of a problem in plantation forests (McNally 1955, Cremer 1969, Fitzgerald 1981). The ecology of the common brushtail possum has been reasonably well documented (e.g. Strahan 1983, Kerle 1984). In forestry terms, damage has been documented in *Eucalyptus* (Cremer 1960, Mollison 1960, Gilbert 1961, Cremer 1969), feeding preferences of brushtails have been investigated for native New Zealand flora (Gilmore 1967), as well as the diet constituents of brushtails in native Tasmanian forest (Fitzgerald 1984). Host preference in *Eucalyptus* has also been investigated within a species (*E. blakelyi*) with respect to irrigated/unirrigated trees (Landsberg 1987) and some host species preferences have been demonstrated in *Pinus* (Barnett *et al.* 1977). However, direct comparisons between *Eucalyptus* species are not available. Some recent unpublished work by Dr. Clare McArthur has demonstrated that species preferences do occur when given choice in feeding trials. In addition, a Tasmanian silvicultural handbook (Nielsen 1990) states that possums prefer *E. globulus* and *E. nitens*. However, even though preferences have been noted in the field, even to a family level (Tibbits pers. comm., Volker pers. comm.), there is only one report with genetic parameter information for browsing damage caused by possums, on *E. nitens* in New Zealand (Cannon 1993).

This study investigates the species preference of brushtail possums in a number of *Eucalyptus* controlled cross experimental field trials in north-west Tasmania. Host species preferences of possums, including the suitability of inter-specific hybrids were able to be addressed directly using these trials, primarily because the eucalypt species were planted in a common environment. In addition, genetic parameters were able to be calculated using the pedigreed material in the trials. The two main eucalypt species used were *E. globulus* Labill. and *E. nitens* (Deane & Maiden) Maiden although 12 other species were included. The damage caused by brushtail possums was then

discussed in relation to the suitability of the eucalypt species examined for intensive forestry.

Materials and methods

Three experimental field trials were assessed for possum damage. All were located near Ridgley, in north-west Tasmania. All trials had an approximate annual rainfall of 1200mm and were planted on a deep, well structured and drained krasnozem soil overlying tertiary basalt.

Trial 1

This trial was established by CSIRO Division of Forestry and North Forest Products. The trial contained progeny from an *E. globulus* factorial, an *E. nitens* half-diallel, an *E. nitens x E. globulus* F_1 hybrid factorial and open-pollinated progeny from the same parents used in these controlled crossing designs. All trial details are given in Chapter 2 , particularly Table 2.1, 2.2 and 2.3; (see also Table 3.1, Volker *et al.* 1994, Hodge *et al.* 1996). The trial contained approximately 6000 trees. Each of the four replicates of 1500 trees was comprised of 15 incomplete blocks. Each incomplete block contained 20 linear plots of 5 trees. The altitude of the trial site was approximately 185m, and spacing between trees was three by four metres.

Trial 2

Progeny from intra- and inter-specific hybrid crosses of *Eucalyptus* were established in an experimental field trial by The University of Tasmania and North Forest Products in September 1988. The main species incorporated in this trial were *E. globulus, E. ovata* Labill., *E. gunnii* J.D.Hook., *E. morrisbyi,* Brett and *E. urnigera* J.D.Hook.. The crosses within and between species which were included in this trial are given in Table 3.2. The origin of the parents and the number of families represented in each provenance are given in Table 3.3. The trial was divided into 5 replicates. Each replicate was divided into 5 blocks to avoid competition between species differing markedly in growth rates. Each block contained three tree linear plots of each family. The subdivision of cross types between blocks was: i) *E. globulus* controlled crosses and open-pollinated families; ii) self-pollinated families of *E. globulus* (always planted adjacent to sub-block i)); iii) *E. ovata* controlled crosses and open-pollinated families; iv) self pollinated families of *E. ovata* (always planted adjacent to sub-block iii) and; v) remaining F_1 hybrids and parental controls. Except for the condition that the self-pollinated families were always planted adjacent to a block of controlled crosses of the same species, the arrangement of the blocks and families within blocks was random. The trial was surrounded by an edge row, which was made up of families similar to families in the adjacent block. Altitude of the site

was approximately 220m and spacing between trees was three by three metres. At the time of assessment the trees were approximately four years old.

Trial 3

The trial was planted adjacent to Trial 2 and was established by North Forest Products in May 1988. The trial contained eight replicates, with families arranged in 5 tree linear plots. Within each replicate, families were randomised, although hybrids and *E. nitens* were grouped separately into blocks to avoid problems of competition. Spacing was 3 x 3 m between trees and both replicates and blocks were surrounded by edge rows where possible. All *E. nitens* progeny were obtained from intra-specific outcrosses. Controls for parents used in the inter-specific crossing were represented in the trial as open-pollinated progeny. Pedigree and provenance information are given in Tables 3.4 and 3.5 respectively. There were approximately 1300 trees within the trial, although a large number of deaths had occurred. The number of families and individuals in each cross type that were assessed for possum damage is given in Table 3.6.

Damage assessment

Possum damage on each individual tree was assessed for all trials using a 6 point qualitative scale, ordered from none or very low (1) to very high (6) (see Table 3.7). Examination of scats demonstrated that the possum species responsible for the damage was *T. vulpecula*. No scats resembling the ringtail possum (*Pseudocheirus peregrinus*) were found. Possum damage is quite distinctive and easily recognised by broken branches, scratches on the trunk and the way the leaf material is removed (see Figure 3.1, (Landsberg 1987). However, in some cases, chrysomelid damage (Leon 1989) may be confounded within the severe damage score but in general this effect was minimal, particularly in Trial 1, where very little chrysomelid damage was apparent. Possum damage was scored in Trial 1 on 8-9th September 1993, in Trial 2 on 19-20th November 1992 and in Trial 3 on 7-9th September 1993.

Data analysis

Trial 1

Genetic parameters and variances were estimated following the data analysis procedures detailed in Chapter 2. Some additional analysis was also undertaken. It was noted, while assessing the hybrid trial for possum damage, that the hybrids had more damage than the other trees. Hybrids were localised within the replicate structure and tended to have a greater number of missing trees per plot than any other cross type. To investigate any possible relationship between increased possum damage and the amount of open space in the immediate vicinity of individual trees (or

tree density), percentage survival was calculated for each plot. All the data were then re-analysed, including all individuals from the controlled-cross hybrids, *E. globulus* factorial and *E. nitens* half-diallel using the percentage plot survivorship as a covariate in REML VCE. All cross types were included to investigate whether tree density had an effect across the different cross types. The model used for these analyses was the same as model [1] in Chapter 2, only with an extra term, the covariate of percentage survival. Further analyses were undertaken within the hybrid and *E. globulus* factorials, and the *E. nitens* half diallel to examine any effect plot survival may have had within a cross type. Cross type was not included in the model for *E. nitens,* as only one cross type within a single provenance was incorporated in the *E. nitens* half-diallel.

Analysis for cross type means

Due to the lack of normality of all the possum damage data ($P<0.001$, Shapiro-Wilk statistic, W) from Trials 1, 2 and 3, arithmetic means of cross types were determined for graphical presentation only, using the MEANS procedure in SAS (SAS 1992). Testing for differences between cross types was then undertaken using the CATMOD procedure in SAS (SAS 1992), and the number of trees in each category (1 to 6) within each cross type. Cumulative logits were chosen as the data were an ordered response. Contrasts between cross types are given as Chi-square values (Wald statistic) and their probabilities.

Results

Host preference

Trial 1

The Taranna (T) provenance of *E. globulus* had significantly higher possum damage than both the King Island (K) provenance and the inter-provenance hybrids (TxK and KxT) ($P<0.001$, see Figure 3.2, Table 3.8). The interspecific hybrids NxT and NxK clearly had the greatest amount of possum damage, with the NxT hybrids being significantly more damaged than the T, K and N populations (Figure 3.2, Table 3.8). Notably, the NxT interspecific hybrids were significantly more damaged than the NxK hybrids, so the greater damage shown in the Taranna provenance when compared with King Island was also detectable when crossed with *E. nitens*. Damage was low on both *E. bicostata* and the *E. bicostata x E. globulus* F_1 hybrids and was not significantly different from damage on the King Island provenance of *E. globulus* or the *E. globulus* inter-provenance hybrids (TxK). All 94 individuals of *E. bicostata* had possum damage scores of 1. This lack of variation therefore meant that it was not

possible to test using CATMOD. Cross type comparisons involving *E. bicostata* were therefore analysed using pairwise contrasts in the NPAR1WAY procedure of SAS (SAS 1992).

Trial 2

Crosses involving *E. morrisbyi* and *E. gunnii* had the greatest possum damage (see Figure 3.3, Table 3.9). Moreover, their F_1 hybrid *E. morrisbyi x E. gunnii* was the most damaged cross type in the trial although it was not significantly more damaged than either of its parent species. The pure species that had very little possum damage included *E. cinerea, E. johnstonii, E. perrinianna, E. ovata, E. urnigera, E. nitens* and *E. globulus* (see Figure 3.3). *E. cordata, E. viminalis* and *E. brookeriana* had intermediate damage levels, whereas *E. gunnii* and *E. morrisbyi* had relatively high levels of possum damage. Those cross types which were well represented in the trial are summarised in Figure 3.4. The general trend, for possum damage on pure species was:

E. morrisbyi =E. gunnii > E. ovata >E. globulus

E. morrisbyi had significantly more possum damage than *E. globulus* (Glo poly, self and GloxGlo, $P<0.001$, Table 3.9). Similarly, *E. morrisbyi* had significantly more possum damage than the *E. morrisbyi x E. globulus* hybrids ($P<0.001$), which in turn had more possum damage than *E. globulus* ($P<0.001$); see Figure 3.4. The damage to *E. morrisbyi x E. globulus* hybrids, was intermediate between the two parent species, consistent with additive inheritance of possum damage.

Almost all hybrids with *E. globulus* had damage levels that were intermediate between the two parent species, although the mean damage levels tended towards the least damaged parent. In particular, *E. globulus* had lower mean possum damage than *E. gunnii x E. globulus* hybrids (See Figure 3.3, 3.4). The mean damage on the *E. nitens x E. globulus* hybrids was slightly higher than the parental controls of *E. nitens* and *E. globulus.* Although this difference was not significant, it was a similar trend to damage on *E. nitens x E. globulus* hybrids determined in Trial 1. Hybrids between other eucalypt species showed similar responses, with mean possum damage intermediate between the corresponding parent species. Hybrids (not including hybrids with *E. globulus*), that showed significant differences when compared with at least one parent species included: *E. gunnii* vs. *E. gunnii x E. johnstonii*, and *E. urnigera* vs. *E. gunnii x E. urnigera,* vs. *E. morrisbyi x E. urnigera.* Both *E. ovata x E. morrisbyi* and the reciprocal cross *E. morrisbyi x E. ovata* were significantly more damaged than *E. ovata.*

In general, when parental species differed significantly in susceptibility, possum damage appeared to be inherited additively, with hybrids nearly always intermediate in damage when compared with parent species.

Trial 3

The amount of possum damage on the five main cross types within this trial is given in Figure 3.5 (*E. globulus*, *E. nitens*, *E. gunnii*, and the F_1 hybrids *E. nitens x E. globulus* and *E. nitens x E. gunnii*), whereas damage on all the cross types within experimental Trial 3 is given in Figure 3.6. The *E. nitens x E. gunnii* F_1 hybrids had intermediate levels of possum damage and were significantly more damaged than *E. nitens* but had significantly less damage compared with *E. gunnii* (Figure 3.5, Table 3.10). The *E. nitens x E. globulus* hybrids were at least as damaged as either parent but these differences were not significant.

E. gunnii and *E. nitens x E. gunnii*, *E. camaldulensis* and *E. nitens x E. camaldulensis* hybrids were the most damaged by possums in experimental Trial 3. The pure species *E. nitens, E. pulverulenta, E. johnstonii, E. cordata* and *E. globulus* had relatively low levels of possum damage. *E. rodwayi, E. morrisbyi, E. camaldulensis* and *E. gunnii* had relatively high levels of possum damage (Figure 3.6). However, differences between species between these categories were often not significant and these were general trends only. Hybrids generally had intermediate damage when compared with their pure parent species. The majority of F_1 hybrids did not have significantly different possum damage when compared with either of their pure parental species, although there were two exceptions: *E. nitens x E. camaldulensis* hybrids were significantly more damaged than *E. nitens* ($P<0.001$); and *E. nitens x E. gunnii* hybrids were significantly more damaged than *E. nitens*, and significantly less damaged than *E. gunnii* ($P<0.001$, Figure 3.5, Table 3.10).

Genetic parameters (Trial 1)

Genetic parameters for possum damage in *E. globulus, E. nitens* and their F_1 hybrid were estimated in Trial 1 and results are given in Table 3.11). Within cross types, *E. globulus* had a very low amount of additive genetic variation for possum damage, with low heritabilities estimated for the controlled- cross material (0.00 to 0.05, Table 3.11). The heritability estimate for the *E. globulus* open-pollinated material was highly inflated when compared with the controlled- cross estimates (0.43 vs 0.00 to 0.05). The proportion of dominance variation was consistantly higher than the heritability estimates and was moderately high for the pooled estimate (0.30). Heritability and dominance estimates for *E. nitens* were very high (0.50 to 0.56). Similarly, the estimate of heritability for the *E. nitens x E. globulus* hybrids was high

(0.70, Table 3.11), although the proportion of dominance variation was relatively low.

Heritabilities estimated with plot survivorship as a covariate were very similar to those estimated without plot survivorship (Table 3.11 vs. Table 3.12). The slopes of plot survivorship, estimated as a fixed effect in VCE REML, were not found to be significant in any of the cross types. (Table 3.12).

Discussion

While results between different field trials may not always be confidently compared, particularly because some species were represented by only a few trees from one provenance while others were well represented, it appeared that the most susceptibile species of those examined were *E. morrisbyi* and *E. gunnii.* The single *E. camaldulensis* family was also highly susceptible. The more resistant species included the major commercial species *E. globulus* and *E. nitens.* In general, the levels of damage followed the order:

E. morrisbyi = *E. gunnii* > *E. ovata* > *E. globulus* = *E. nitens*

While the comparisons of possum damage on poorly represented species is useful, it is important to realise that in all the experimental field trials there were either low number of families or a low number of provenances representing these species. It would be unwise to extrapolate some of the results to the species level. In Trial 2, the juvenile-persistant species *E. pulverulenta, E. cordata* and *E. perriniana* included trees with juvenile rather than adult foliage. Hence foliage type may have been confounded to some extent for these species although these comparisons are probably biologically significant. However, in the majority of cases, possum damage referred directly to damage on adult foliage.

The extent of possum damage on interspecific F_1 hybrids was generally intermediate when compared with the damage levels on either parent species. Possum damage therefore appeared to be inherited predominantly in an additive manner in inter-specific crosses. There was only one instance where the hybrids had greater possum damage than either pure parental species. The hybrids *E. nitens x E. globulus*, from experimental Trial 1 were significantly more damaged than either parent species. However, only one *E. nitens* provenance was represented in this trial (Toorongo) compared with 4 provenances in experimental Trial 3, (see Table 3.1). In addition, only two provenances of *E. globulus* were represented. In both of the other trials

investigated, damage on these cross types was proportionately higher and the interspecific F_1 hybrid was not significantly different from either of the parent species.

In the *E. nitens x E. globulus* hybrid trial (Trial 1), the F_1 hybrids were significantly more susceptible than either parent species. The hybrids between these two species were not found to be significantly more damaged than the parent species in the other two trials investigated (Trial 2 and 3) although the mean possum damage of the hybrids was slightly greater than either *E. globulus* or *E. nitens* in all cases. This result adds some confidence to the hybrid susceptibility noted in Trial 1. In Trials 2 and 3, tree species (e.g. *E. morrisbyi*) more susceptible to possum damage than *E. globulus* or *E. nitens* were present. It is possible that planting more susceptible species in a common environment may have masked the preference between *E. globulus*, *E. nitens* and their hybrids due to the lower damage levels they incurred, whereas, in Trial 1 no susceptible trees were growing in the immediate vicinity.

Very little genetic variation was detected for possum damage within the separate *E. globulus* provenances, although a significant difference between damage levels was noted between the King Island and Taranna provenances. Damage levels were very low, however and it is possible that heritability estimates would be larger with more damage (White and Hodge 1989). In contrast, considerable genetic variation was detected within the Toorongo provenance of *E. nitens* in the same field trial, with a heritability of 0.40 (controlled-cross estimate). Heritability estimates obtained for possum defoliation on *E. nitens* in New Zealand (0.30 and 0.38, Cannon 1993) were comparable with those obtained here. It appears that, at least in *E. nitens,* there is sufficient genetic variation to enable breeding for resistance to possum damage. However, the damage levels determined on *E. nitens* in all three experimental trials were not very high and it is possible that breeding for possum resistance in *E. nitens* may not be necessary for sites in Tasmania.

Analysis of the data including percentage plot survival as a covariate gave very similar heritabilites and proportion of dominance variance estimates. Given that the slope of the covariate was not significantly different from zero in all cases tested, it appears that the plot survivorship was not important in affecting host choice within experimental field Trial 1. Hence it appears that whether the area in the immediate vicinity was more open or not was not important in the host choice made by the possums.

The proportion of dominance variation for possum damage was low in both the separate *E. globulus* provenances (0.08 to 0.17) and the F_1 hybrids between *E. nitens* and *E. globulus* (0.03). Dominance variation for *E. nitens* and *E. globulus* (pooled) was moderate to high (0.54 and 0.30 respectively). Dominance can contribute to

inflation of open-pollinated estimates and this may have contributed to the inflated open-pollinated heritability estimate for *E. globulus*. The high dominance variation, reflecting the susceptibility of specific families, suggests that a high proportion of resistance to possum damage may be governed by a few major genes in *E. globulus* and *E. nitens*.

E. nitens and *E. globulus* are two of the most important eucalypts in temperate hardwood forestry (Eldridge *et al.* 1994). Results obtained suggest that they are also some of the most resistant species to possum damage. However, in the absence of more susceptible species, both *E. nitens* and particularly the *E. nitens x E. globulus* F_1 hybrids are significantly more damaged than *E. globulus*. Hence in areas where possum damage may be a problem, *E. globulus* would be the best species choice at least when compared with the Toorongo provenance of *E. nitens*. It is possible that co-planting *E. nitens* with more susceptible species may be a future option (e.g. *E. gunnii*), since *E. globulus* is limited by altitude in Tasmania (Tibbits 1986).

Table 3.1. The number of families and individuals of each cross type in field Trial 1 near West Ridgley. (OP = open-pollinated). Provenances are in brackets following species names. *E. globulus* pooled contains the *E. globulus* inter- and intra-provenance crosses between Taranna (T) and King Island (K); (T, K, TxK and KxT, see Table 3.2).

Cross type	number of families	number of individuals
E. globulus pooled	168	3486
E. globulus (King Island)	28	579
E. globulus (Taranna)	55	1096
E. globulus OP	25	516
E. globulus seed orchard OP	8	155
E. nitens (Toorongo)	36	714
E. nitens seed orchard OP	9	194
E. nitens x E. globulus F_1 hybrid	43	665

Table 3.2 Crossing diagram for experimental Trial 2. Numbers indicate the number of individuals of each cross type within the trial.

		male parent														
		Arch	Bro	Cin	Cord	Glo	Gpoly	Gun	John	Mor	Ov	Perr	Urn	Vim	op	self
f	Bro					6									8	
e	Cin														12	
m	Cord														4	
a	Glo					210	150	6					20		369	116
l	Gun					37		52	16				10		139	10
e	John														11	
	Mor	24	17			54		10	25	33	46		47	17	41	
p	Nit					8									6	
a	Perr														5	
r	Pulv														4	
e	Urn														24	4
n	Vim														27	
t	Ov		24	16	33	127	16		8	21	114		25	22	264	35

Note: Arch= *E. archeri*, Bro = *E. brookeriana*, Cin = *E. cinerea*, Glo = *E. globulus*, Gpoly = *E. globulus* polymix, Gun = *E. gunnii*, John = *E. johnstonii*, Mor = *E. morrisbyi*, Ov = *E. ovata*, Perr = *E. perriniana*, Urn = *E. urnigera*, Vim = *E. viminalis*, op = open pollinated, self = self pollinated.

Table 3.3. Origin of parents used in crosses growing in Trial 2. A large number of parents were from exotic plantings in gardens and on roadsides. These were of unknown provenance.

parent species	total parents	provenance (no. parents)
E. brookeriana	2	King Island (1), Unknown (1)
E. camaldulensis	1	Unknown (1)
E. cinerea	1	Unknown (1)
E. cordata	2	Snug Plains (1), Unknown (1)
E. globulus	12	Proctors Rd (4), Tinderbox (1), Unknown (4), King Island (2), Bruny Island (1).
E. gunnii	8	Shannon Lagoon (4), Todd's corner (1), Snug Plains (3)
E. johnstonii	1	The Springs, Mt. Wellington (1)
E. ovata	12	Harris Creek (1), Kingston (8), Southern Outlet (3)
E. morrisbyi	2	Calvert's Hill (2)
E. nitens	2	Toorongo (CSIRO, 2)
E. perriniana	1	Ornamental in View St, Sandy Bay
E. pulverulenta	1	Unknown (1)
E. urnigera	3	The Springs, Mt. Wellington (3)
E. viminalis	1	Calvert's Hill (1)

Table 3.4. Pedigree of crosses incorporated in Trial 3, near West Ridgley. All hybrids had *E. nitens* as the female parent and all pure species controls were open-pollinated (the male parent was unknown) except for *E. nitens*.

species	code	female	male	hybrid cross	code	female	male
E. camaldulensis	CAM	CAM1	open pollinated	x*E. camaldulensis*	XCAM	NIT1	CAM1
E. cordata	COR	COR1	open pollinated			NIT2	CAM1
E. globulus	GLO	GLO1	open pollinated			NIT3	CAM1
		GLO2	open pollinated			NIT7	CAM1
		GLO3	open pollinated			NIT5	CAM1
		GLO4	open pollinated	x*E. cordata*	XCOR	NIT7	COR1
E. gunnii	GUN	GUN1	open pollinated	x*E. globulus*	XGLO	NIT1	GLO5
		GUN2	open pollinated			NIT2	GLO1
		GUN3	open pollinated			NIT3	GLO2
		GUN4	open pollinated			NIT7	GLO2
		GUN5	open pollinated			NIT5	GLO3
		GUN6	open pollinated			NIT7	GLO3
E. johnstonii	JOH	JOH1	open pollinated			NIT8	GLO3
E. morrisbyi	MOR	MOR1	open pollinated			NIT9	GLO3
E. pulverulenta	PUL	PUL1	open pollinated			NIT5	GLO4
		PUL2	open pollinated			NIT6	GLO4
E. rodwayi	ROD	ROD1	open pollinated			NIT7	GLO4
E. viminalis	VIM	VIM1	open pollinated			NIT9	GLO4
E. nitens	NIT	NIT1	NIT4	x*E. gunnii*	XGUN	NIT7	GUN5
		NIT2	NIT10			NIT8	GUN6
		NIT3	NIT11			NIT11	GUN1
		NIT4	NIT1			NIT1	GUN1
		NIT5	NIT9			NIT3	GUN1
		NIT6	NIT9			NIT4	GUN1
		NIT7	NIT9			NIT9	GUN3
		NIT8	NIT9			NIT5	GUN4
		NIT9	NIT5			NIT6	GUN4
						NIT9	GUN2
				x*E. johnstonii*	XJOH	NIT11	JOH1
				x*E. morrisbyi*	XMOR	NIT11	MOR1
				x*E. ovata*	XOV	NIT11	OVA1
				x*E. perriniana*	XPER	NIT11	PER1
				x*E. pulverulenta*	XPUL	NIT1	PUL1
						NIT2	PUL1
						NIT1	PUL2
				x*E. rodwayi*	XROD	NIT11	ROD1
				x*E. viminalis*	XVIM	NIT11	VIM1
						NIT11	VIM2

Table 3.5. Provenance information for parents used in Trial 3.

parent	provenance	parent	provenance
NIT1	North NSW	CAM1	Unknown
NIT2	Toorongo	COR1	Unknown
NIT3	South NSW	GLO1	Geeveston
NIT4	Errinundra	GLO2	Leprena
NIT5	Unknown	GLO3	Otway
NIT6	Unknown	GLO4	North Flinders Ild.
NIT7	Unknown	GUN1	Pensford
NIT8	Unknown	GUN2	Pensford
NIT9	Unknown	GUN3	Central Plateau
NIT10	Toorongo	GUN4	Central Plateau
NIT11	Macalister	GUN5	Central Plateau
VIM1	Unknown	GUN6	Central Plateau
VIM2	Unknown	JOH1	Mt. Wellington
ROD1	Steppes	PUL1	Unknown
MOR1	Cremorne	PUL2	Unknown

Table 3.6. The number of individuals (n) and number of families (n [families]) in each cross type in Trial 3. See also Table 3.4 for pedigree information.

cross	cross code	n	n (families)
E. camaldulensis	CAM	7	1
E. cordata	COR	5	1
E. globulus	GLO	42	4
E. gunnii	GUN	113	6
E. johnstonii	JOH	19	1
E. morrisbyi	MOR	9	1
E. pulverulenta	PUL	3	1
E. rodwayi	ROD	8	1
E. nitens	NIT	164	8
E. nitens x E. camaldulensis	XCAM	56	5
E. nitens x E. globulus	XGLO	64	8
E. nitens x E. gunnii	XGUN	129	9
E. nitens x E. johnstonii	XJOH	13	1
E. nitens x E. morrisbyi	XMOR	27	1
E. nitens x E. pulverulenta	XPUL	25	3
E. nitens x E. rodwayi	XROD	8	1
E. nitens x E. viminalis	XVIM	28	2

Table 3.7. Values and description of the damage for the qualitative score used in the assessment of possum damage in the experimental field Trials.

score	description of damage
1	No damage or very little damage discernable
2	Some damage -a few eaten leaves and/or scratch marks on trunk.
3	Damage immediately obvious, 20% < damage<50% of crown foliage removed, scratch marks evident on trunk.
4	Damage immediately obvious, but ≥50% and <65% damage to crown. Scratch marks evident on trunk.
5	Damage fairly serious, at least 65% of leaf material lost, but less than 80%. Leaf material still obvious in the crown and scratch marks on trunk. Some broken branches may be present.
6	Serious damage, with at least 80% of leaf material lost, scratch marks on trunk very obvious and branches usually broken in the canopy.

Table 3.8. Probabilities of pair-wise tests undertaken for the different cross types within the hybrid trial at West Ridgley. Analysis was undertaken using the CATMOD procedure and probabilities were obtained from Chi-square values (Wald statistic). Crosses are: Taranna (T) and King Island (K) provenances of *E. globulus*, inter-provenance crosses within *E. globulus* (TxK, incorporating both TxK and KxT crosses), *E. nitens* (N), interspecific crosses between *E. nitens* and *E. globulus* (NxT and NxK), *E. bicostata* (B) and interspecific crosses between *E. bicostata* and *E. globulus* (BxG).

	T	K	TxK	NxT	NxK	N	B	BxG
T								
K	0.000							
TxK	0.000	0.107						
NxT	0.000	0.000	0.000					
NxK	0.000	0.000	0.000	0.011				
N	0.000	0.000	0.000	0.000	0.000			
B	0.007	0.313	0.180	0.000	0.000	0.000		
BxG	0.052	0.984	0.462	0.000	0.000	0.002	0.997	

Table 3.9. Pairwise comparisons between all the cross types within experimental Field Trial 2. All contrasts were undertaken using the CATMOD procedure in SAS (SAS 1992) n.s. = not significant.

	Bro op	Cin op	Cord op	Glo	Gun	John op	Mor	Nit op	Perr op	Pulv op	Urn op	Vim op	Ov	Bro x Glo	Glo x Gun	Glo x Urn	Gun x Glo	Gun x John	Gun x Urn	Mor x Arch	Mor x Bro	Mor x Glo	Mor x Gun	Mor x Urn	Mor x John	Mor x Ov	Mor x Vim	Nit x Glo	Ov x Bro	Ov x Cin	Ov x Cord	Ov x Glo	Ov x Gpuly	Ov x John	Ov x Urn	Ov x Vim
Cin op	n.s.																																			
Cord op	n.s.	n.s.																																		
Glo	0.002	n.s.	0.015																																	
Gun	n.s.	n.s.	n.s.	0.000																																
John op	n.s.	n.s.	n.s.	n.s.	n.s.																															
Mor	n.s.	n.s.	n.s.	0.000	n.s.	n.s.																														
Nit op	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.																													
Perr op	n.s.	n.s.	n.s.	0.027	n.s.	n.s.	n.s.	n.s.																												
Pulv op	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.																											
Urn op	n.s.	n.s.	n.s.	0.000	0.000	n.s.	0.000	n.s.	n.s.	n.s.																										
Vim op	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.002																									
Ov	n.s.	n.s.	n.s.	0.000	0.000	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	0.018																								
Bro x Glo	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.																							
Glo x Gun	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.																						
Glo x Urn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.																					
Gun x Glo	n.s.	n.s.	n.s.	0.020	0.000	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	0.000	0.006	0.016	0.035	n.s.																				
Gun x John	n.s.	n.s.	n.s.	0.004	0.014	n.s.	0.004	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.																			
Gun x Urn	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.045	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.																		
Mor x Arch	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.004	n.s.	0.034	n.s.	n.s.	n.s.	0.000	n.s.	n.s.																	
Mor x Bro	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.003	n.s.	0.020	n.s.	n.s.	n.s.	0.000	0.038	n.s.	n.s.																
Mor x Glo	n.s.	n.s.	n.s.	0.000	0.008	n.s.	0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.006	n.s.	n.s.	n.s.	n.s.														
Mor x Gun	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.008	n.s.	0.014	n.s.	n.s.	n.s.	0.001	0.041	n.s.	n.s.	n.s.	n.s.														
Mor x Urn	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.001	n.s.	0.043	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.													
Mor x John	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.016	n.s.	n.s.	n.s.	n.s.	n.s.	0.002	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.												
Mor x Ov	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.000	n.s.	0.000	n.s.	n.s.	n.s.	0.000	0.010	n.s.	n.s.	n.s.	0.010	n.s.	n.s.	n.s.											
Mor x Vim	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.002	n.s.	0.012	n.s.	n.s.	n.s.	0.000	0.025	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.										
Nit x Glo	n.s.	n.s.	n.s.	0.634	0.012	n.s.	0.005	n.s.	n.s.	n.s.	n.s.	0.023	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.043	0.025	0.018	n.s.	n.s.	0.027	0.039	0.009	0.013									
Ov x Bro	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	0.014	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.025	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.							
Ov x Cin	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	0.036	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.039	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.041	n.s.	n.s.	n.s.						
Ov x Cord	n.s.	n.s.	n.s.	0.000	0.033	n.s.	0.006	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.011	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.027	n.s.	n.s.	n.s.	n.s.					
Ov x Glo	n.s.	n.s.	n.s.	0.000	0.000	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	0.004	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.008	0.005	n.s.	0.014	0.002	0.031	0.000	0.003	n.s.	n.s.	n.s.	n.s.				
Ov x John	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			
Ov x Urn	n.s.	n.s.	n.s.	n.s.	0.012	n.s.	0.003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.042	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
Ov x Vim	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.031	n.s.	n.s.	n.s.	n.s.	n.s.	0.004	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Ov x Mor	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.001	n.s.	0.004	n.s.	n.s.	n.s.	0.000	0.019	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.011	n.s.	n.s.	n.s.	0.001	n.s.	n.s.	0.026	n.s.

Table 3.10. Pairwise contrasts between different cross types in experimental Trial 3. Significant differences at least at the 0.05 level are given in bold. Contrasts were determined using the CATMOD procedure in SAS (SAS 1992) and probabilites were obtained from Chi-square Wald statistics.

	CAM	COR	GLO	GUN	JOH	MOR	PUL	ROD	NIT	XCAM	XCOR	XGLO	XGUN	XJOH	XMOR	XOVA	XPUL	XROD
COR	0.54																	
GLO	0.18	0.66																
GUN	0.07	**0.02**	**0.00**															
JOH	0.32	0.86	0.69	**0.00**														
MOR	0.74	0.71	0.26	**0.01**	0.47													
PUL	0.41	0.74	0.93	**0.04**	0.80	0.52												
ROD	0.81	0.71	0.35	**0.05**	0.53	0.96	0.53											
NIT	0.11	0.54	0.69	**0.00**	0.46	0.14	0.99	0.26										
XCAM	0.58	0.76	0.09	**0.00**	0.40	0.86	0.54	0.85	**0.01**									
XCOR	0.37	0.93	0.57	**0.00**	0.89	0.54	0.75	0.59	0.35	0.50								
XGLO	0.37	0.99	0.30	**0.00**	0.74	0.56	0.69	0.62	0.06	0.43	0.87							
XGUN	0.68	0.66	**0.02**	**0.00**	0.26	0.99	0.48	0.96	**0.00**	0.69	0.34	0.18						
XJOH	0.18	0.59	0.80	**0.00**	0.60	0.26	0.98	0.33	0.96	0.18	0.51	0.36	0.11					
XMOR	0.54	0.83	0.20	**0.00**	0.52	0.79	0.58	0.80	0.06	0.87	0.63	0.64	0.63	0.26				
XOVA	0.69	0.80	0.39	**0.02**	0.60	0.92	0.58	0.89	0.28	0.98	0.67	0.71	0.89	0.36	0.92			
XPUL	0.37	0.95	0.49	**0.00**	0.85	0.54	0.72	0.60	0.25	0.48	0.96	0.90	0.29	0.46	0.62	0.68		
XROD	0.50	0.97	0.55	**0.00**	0.80	0.70	0.70	0.71	0.40	0.74	0.88	0.96	0.61	0.49	0.83	0.80	0.90	
XVIM	0.54	0.83	0.21	**0.00**	0.53	0.79	0.59	0.80	0.07	0.87	0.64	0.65	0.64	0.26	0.99	0.92	0.63	0.83

NOTE: COR =*E. cordala*, GLO =*E. globulus*, GUN =*E. gunnii*, JOH =*E. johnstonii*, MOR =*E. morrisbyi*, PUL =*E. pulverulenta*, ROD =*E. rodwayi*, NIT =*E. nitens*, XCAM =*E. nitens x E. camaldulensis*, XCOR =*E. nitens x E. cordata*, XGLO =*E. nitens x E. globulus*, XGUN =*E. nitens x E. gunnii*, XJOH =*E. nitens x E. johnstonii*, XMOR =*E. nitens x E. morrisbyi*, XPUL =*E. nitens x E. pulverulenta*, XROD =*E. nitens x E. rodwayi*, XVIM =*E. nitens x E. viminalis*.

Table 3.11. Individual narrow sense heritibilities (h^2) and proportion of dominance variation (d^2), calculated from controlled cross and open-pollinated families for *E. globulus* and *E. nitens* and their F_1 hybrid. All estimates were calculated using REML VCE (Groeneveld 1995) and standard errors for heritabilities were calculated following (Becker 1985). (N/E = not estimatible).

	$h^2 \pm se$	d^2
E. globulus		
pooled factorial	0.04 ±0.02	0.30
King Island only	0.00 ±0.00	0.17
Taranna only	0.05 ±0.03	0.08
Open-pollinated	0.43 ±0.13	N/E
E. nitens		
half-diallel	0.54 ±0.11*	0.54*
open-pollinated	0.56 ±0.20	N/E
E. nitensx E. globulus		
factorial	0.72 ±0.11	0.03

* Note: the h^2 and d^2 values obtained for *E. nitens* half-diallel add to a value greater than one. This can occur because of sampling error and is indicated partially by the moderately high standard error for the h^2 of this cross type.

Table 3.12. Individual narrow sense heritibilities (h^2) and proportion of dominance variation (d^2), calculated from controlled cross and open-pollinated families for *E. globulus* and *E. nitens* and their F_1 hybrid with the percentage survivorship per plot as a covariate. Slope of the covariate and the probability of the test that the slope is significantly different from zero (Student's t-test) is also given. All estimates were calculated using REML VCE (Groeneveld 1995) and standard errors were calculated following (Becker 1985). (N/E = not estimatible).

cross	**$h^2 \pm se$**	**d^2**	**slope of covariate**	**P**
E. globulus				
pooled factorial	0.04±0.02	0.31	-0.001±0.001	P>0.50
Open-pollinated	0.44±0.10	N/E	0.001±0.003	P>0.50
E. nitens				
half-diallel	0.40±0.09	0.56	-0.021±0.006	P>0.50
open-pollinated	0.63±0.20	N/E	0.002±0.016	P>0.50
E. nitensx E. globulus				
factorial	0.72±0.11	0.04	0.001±0.003	P>0.50

Figure 3.1. Damage caused by possums in the field: characters used in the qualitative scoring system (see Table 5.7).

i) Characteristic broken branches and leaf material loss

ii) Scratches caused by possums on an *E. nitens* trunk.

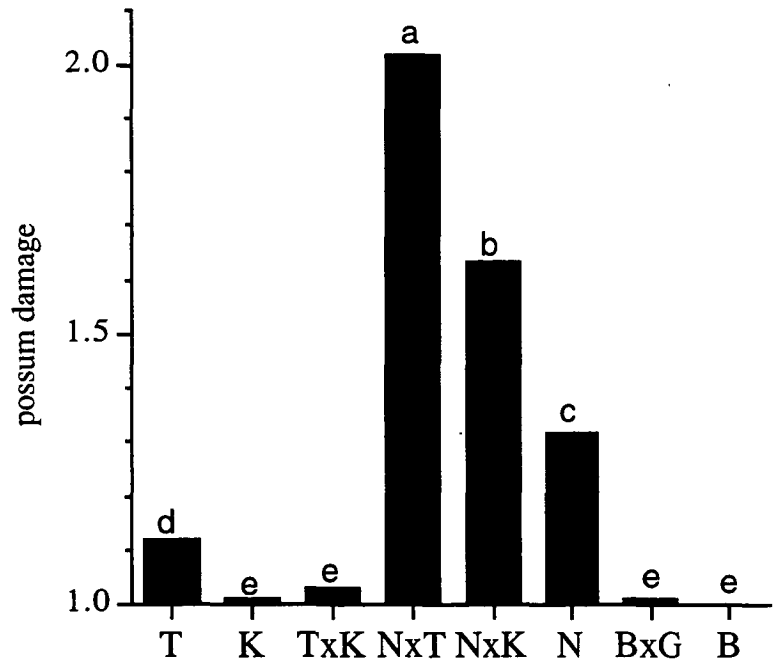
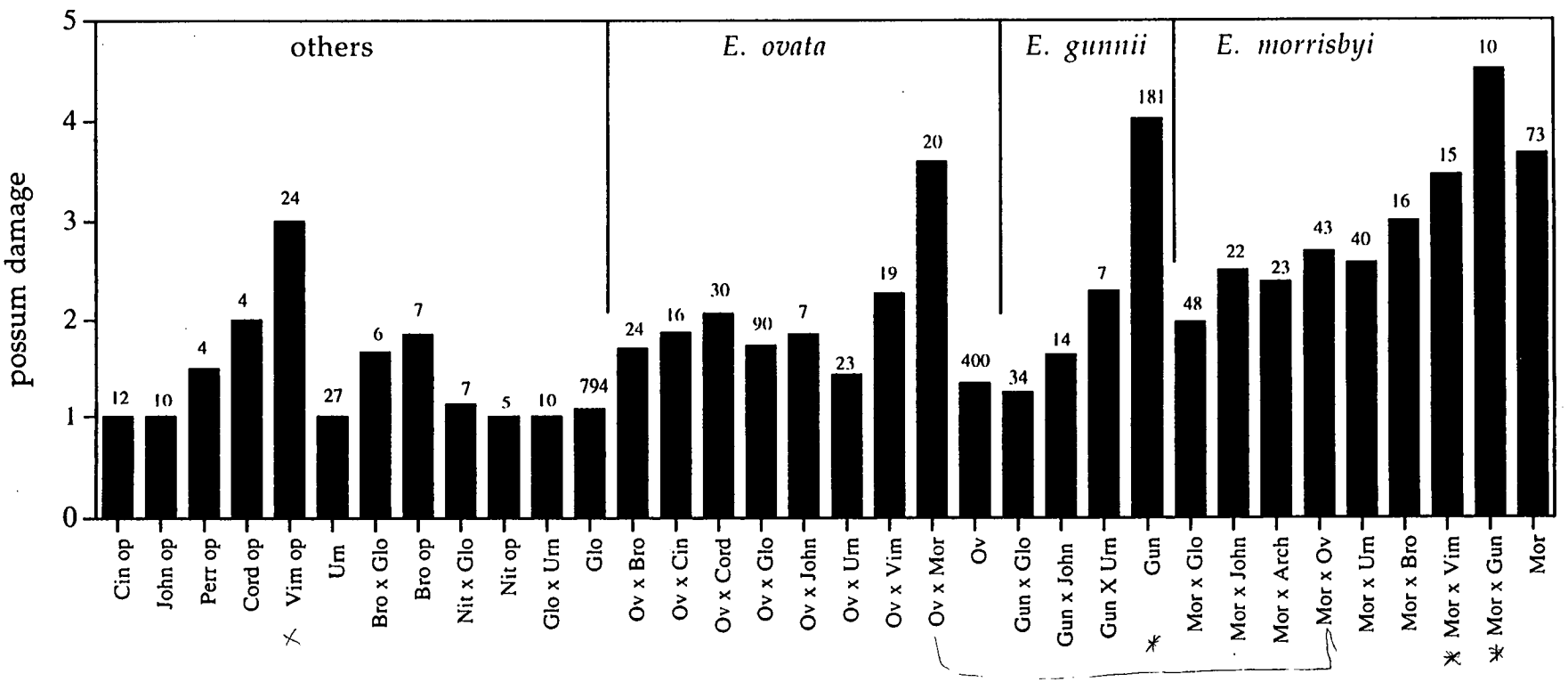


Figure 3.2. Mean possum damage score on the different cross types within experimental field Trial 1. High damage is represented by a high value (1 to 6). Cross types included the Taranna (T) and King Island (K) provenances of *E. globulus*, inter-provenance crosses within *E. globulus* (TxK, incorporating both TxK and KxT crosses), *E. nitens* (N), interspecific crosses between *E. nitens* and *E. globulus* (NxT and NxK), *E. bicostata* (B) and interspecific crosses between *E. bicostata* and *E. globulus* (BxG). Different letters above the cross types (except *E. bicostata*) represent significant differences at the 0.05 level, given by the Chi-square test (Wald statistic) using the CATMOD procedure of SAS (SAS 1992). Cross type comparisons involving *E. bicostata* were determined using the NPAR1WAY procedure of SAS (SAS 1992). Specific pair-wise comparison of cross types are given in Table 3.8.

Figure 3.3. Means of possum damage for cross types in Trial 2, where n≥4 within experimental Trial 2. Crosses involving *E. morrisbyi* are grouped on the right hand side, crosses involving *E. gunnii* and *E. ovata* are also grouped as indicated. Other cross types (others) included pure species controls and other hybrid cross types. Numbers above the columns indicate sample size for specific cross types. The cross type Mor included both MorxMor and Mor op. Similarly, Gun included Gun op, GunxGun and Gun self; Ov included Ov self, Ov poly and Ov op crosses and; Urn included pooled Urn self and Urn op. See Table 3.4 for species codes and pedigrees. Specific pairwise contrasts are given in Table 3.9.



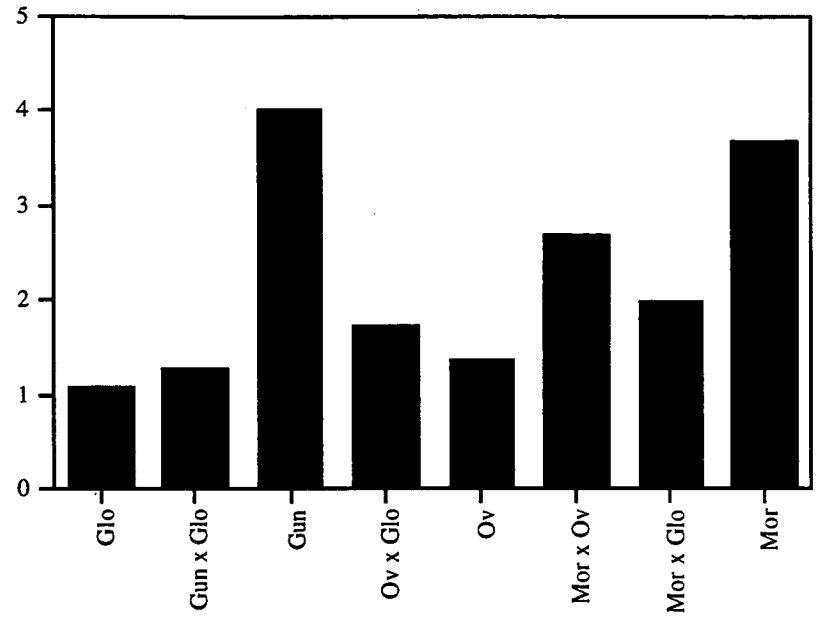


Figure 3.4. Mean possum damage on the cross types well represented in Trial 2. Significant differences between cross types are given in Table 3.9 and were obtained from pairwise contrasts using the CATMOD procedure of SAS (SAS 1992).

Figure 3.5. Mean possum damage on the cross types well represented in experimental Trial 3, *E. globulus* (GLO), *E. gunnii* (GUN), *E. nitens* (NIT) and the *E. nitens x E. globulus* (XGLO) and *E. nitens x E. gunnii* (XGUN) hybrids. Different letters above the different cross types represent significant differences at the 0.05 level (Chi-square test, Wald statistic, see also Table 3.10).

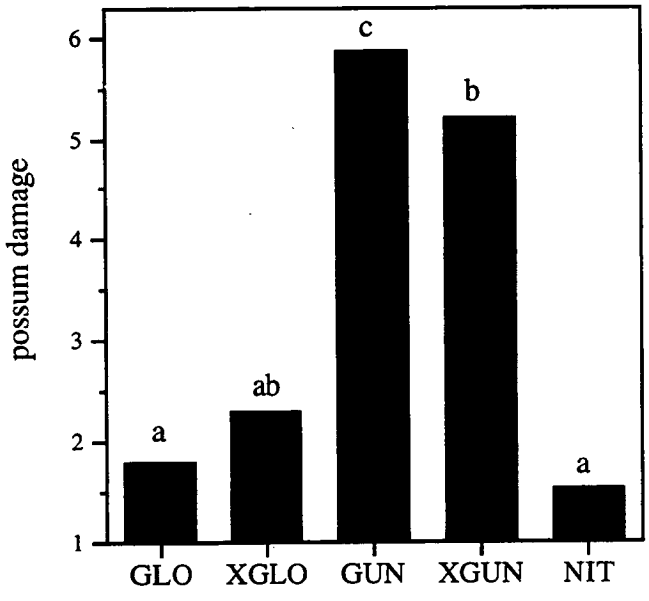
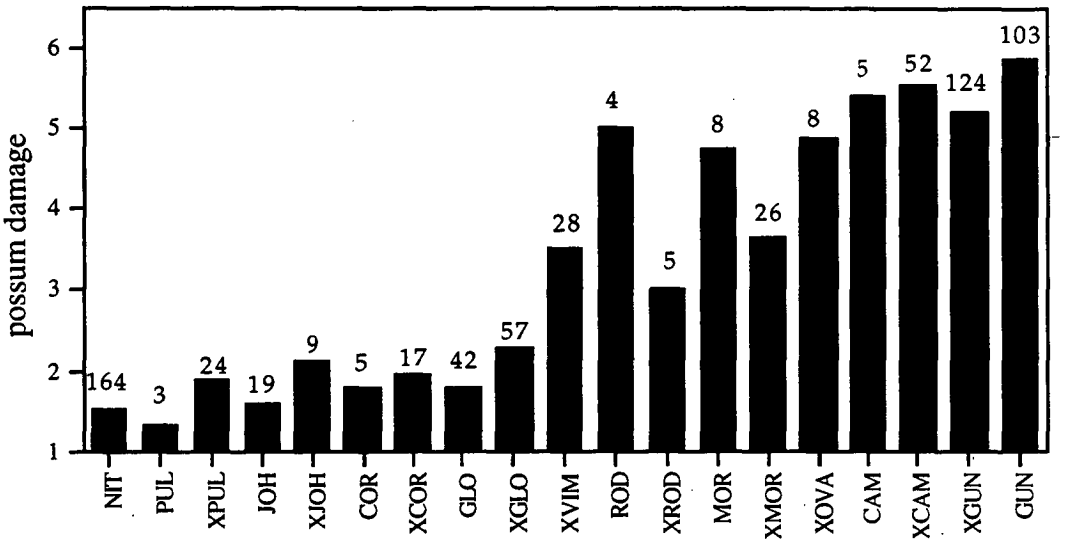


Figure 3.6. Mean possum damage on all the cross types within Trial 3. Numbers above cross types indicates the number of individuals within each cross type. Pairwise contrasts between cross types are given in Table 3.10. In the majority of cases, the F_1 hybrid is paired with the open-pollinated family from the male parent used in the cross (PUL, JOH, COR, ROD, MOR, CAM).



NOTE: NIT = *E. nitens*, PUL = *E. pulverulenta*, XPUL = *E. nitens* x *E. pulverulenta*, JOH = *E. johnstonii*, COR = *E. cordata*, XCOR = *E. nitens* x *E. cordata*, GLO = *E. globulus*, XGLO = *E. nitens* x *E. globulus*, XVIM = *E. nitens* x *E. viminalis*, ROD= *E. rodwayi*, XROD= *E. nitens* x *E. rodwayi*, MOR = *E. morrisbyi*, XMOR = *E. nitens* x *E. morrisbyi*, XOVA = *E. nitens* x *E. ovata*, XCAM = *E. nitens* x *E. camaldulensis*, XGUN = *E. nitens* x *E. gunnii*, GUN = *E. gunnii*

Chapter 4

Leaf area loss caused by chrysomelids and *Uraba lugens*, host preference and hybrid susceptibility

Introduction

There has been considerable debate on the extent of leaf area loss from insect defoliation in eucalypt forests (Ohmart 1984, Fox and Morrow 1986, Ohmart and Edwards 1991 and references therein). Leaf area loss in eucalypts has been quoted as being as high as 50% in Australian forests (see Fox and Morrow 1983, Ohmart *et al.* 1983, Ohmart 1984, Fox and Morrow 1986, Ohmart and Edwards 1991). This level of defoliation is higher than estimates for northern hemisphere forests (see Ohmart 1984, Fox and Morrow 1986, Ohmart and Edwards 1991). However, the methods used for defoliation assessment varies and is often subjective (see Landsberg 1989) and rates of herbivory have been shown to increase with increasing site productivity (Landsberg and Gillieson 1995). It is therefore not easy or even correct to compare leaf area loss both between methods and between sites, even though some indication of leaf area loss is given (see Ohmart and Edwards 1991, Landsberg and Gillieson 1995 and references therein). However, there is considerable interest in the effect of leaf area loss on production in the commercial forests and possible economic losses caused by such potentially high loss of productive photosynthetic area (e.g. Leon 1989, Elliot *et al.* 1993).

In the Southern Australian states, chrysomelid beetles are important defoliators of eucalypts (Elliot *et al.* 1993). The extent of defoliation resulting from these beetles has been estimated to be as high as 40% (Kile 1974) and has significantly reduced height growth (Leon 1989, Elliot *et al.* 1993). The economic losses caused by chrysomelids is of major importance to the forest production and has warranted the development of an integrated pest management strategy in Tasmania (Elliot *et al.* 1992, Greener and Candy 1994).

In Tasmania, the main chrysomelid defoliator species include *Paropsis porosa*, *P. charybdis*, *Chrysophtharta bimaculata*, and *C. agricola* (deLittle 1989). While all these species contribute to leaf area loss in eucalypts, it is the leaf beetle *C. bimaculata* that is singularly responsible for most severe defoliation of young eucalypts in Tasmania (Greaves 1966, Kile 1974, deLittle 1989, Leon 1989, Elliot *et al.* 1992, Elliot *et al.* 1993). Another low level but persistent pest of plantations is the gum leaf skeletoniser, *Uraba lugens* (Harris 1975, Howes 1990). Attacks by *U.*

lugens rarely kill mature trees, but young trees can be sufficiently damaged to be killed (Elliot and deLittle 1984). Severe outbreaks of this insect in native bushland have been reported, and this insect has the potential for becoming a significant pest in commercial forests (Campbell 1962).

This study investigates the species preference of chrysomelid beetles and of the gum leaf skeletoniser *Uraba lugens* in a pedigreed *Eucalyptus* field trial in north-west Tasmania. In total, over 15 species of eucalypt were investigated, including *E. globulus*, *E. nitens*, *E. gunnii*, *E. ovata* and *E. morrisbyi*. Interspecific hybrids between *E. nitens* and a large number of the other eucalypt species, also present in the field trial, were investigated in terms of their susceptibility to chrysomelids and the gum leaf skeletoniser relative to their parent species. In addition, the quantitative leaf area loss on *E. nitens*, *E. globulus* and their F_1 hybrid is determined.

Materials and Methods

Plant material

Trees studied were planted in a hybrid trial that was established near West Ridgley in north-west Tasmania in May 1988 by North Forest Products. The trial incorporated approximately 1300 trees, with 12 different eucalypt species and a large number of F_1 hybrids with *E. nitens* as the female parent. The trial was arranged in eight replicates, with like cross types (e.g. hybrids) blocked within replicates to avoid competition. Within blocks, trees were arranged in 5 tree linear plots. Spacing was 3x3 metres. Detailed information is given in Chapter 3 (Trial 3).

Qualitative damage assessment

Damage caused by chrysomelids and the gum leaf skeletoniser *Uraba lugens* was assessed on each individual tree in the field trial. Qualitative assessment was undertaken using a qualitative 6 point scale, given in Table 4.1 similar to the approach taken by Raymond (1995) and based on prior visual assessment of the range of damage within the trial. Defoliation caused by chrysomelids ranged from none or very little, to almost complete destruction of the new season's canopy. *U. lugens* damage also ranged from none to a severely affected canopy. Chrysomelid damage was scored on the 10th of February 1993 and the 4th of April 1993. *U. lugens* damage was scored on the 4th of April 1993.

These data failed to satisfy the normality assumptions of analysis of variance so a categorical model using the number of individual trees within each category was analysed using the CATMOD procedure of SAS (1992). Cumulative logits were chosen and the response function was included in the model with cross type. Specific pair-wise comparisons were undertaken using the contrast option, which gave Chi-square values and their corresponding probabilities based on the Wald statistic (for data sets less than 2000, SAS (1992). Cross type means (for graphical purposes), were obtained using the MEANS procedure in SAS (1992).

Quantitative leaf area loss

The eucalypts used in this experiment were planted in the field trial described above and in Chapter 3 (Trial 3). A subset of the trial was sampled, including three *E. globulus*, four *E. nitens* and five *E. nitens x E. globulus* hybrid families (see Table 4.2). All *E. globulus* parents within the subset sampled were represented as open-pollinated progeny collected from a seedling seed orchard in north-west Tasmania. Similarly, all *E. nitens* parents were controlled-cross progeny also from a seedling seed orchard in north-west Tasmania (see also details of Trial 3, Chapter 3). At the time of sampling, the trees were approximately 6 years old and 12 metres in height. The trees used in this experiment were felled for wood density measurements by North Eucalypt Technologies. This enabled full access to the canopy of all the trees. five trees per family of each of the 12 families were felled, and 10 branchlet samples per tree were collected from within the trial. Of these 10 samples, 5 were collected randomly from the upper third of the canopy and 5 were collected randomly from the lower two thirds of the canopy. The distinction between the upper third and lower two thirds of the canopy was made because leaf flush in the upper third was more aggressive and therefore had a different structure. Samples consisted of branchlets of approximately 40 cm in length, measured along the main stem. Along the main stem, the number of nodes, the number of leaves and hence the number of leafless nodes were determined. All leaf samples were collected in late April 1994 and were of the adult foliage type. Traits scored and derived from characters scored from all samples are given in Table 4.3.

The leaves on the main stem were divided into two groups: those resulting from the current seasons growth (1993-4, new), and those resulting from the previous seasons growth (pre-1993-4, old). The distinction between these two groups was made by the length of internodes (very small in winter growth) and stem colour (greener in new season's growth). Actual leaf area for new and old leaves on the main stem were measured using a leaf area meter (Delta -T Devices Cambridge, U.K.). Extrapolated leaf areas were estimated from the actual leaf area by drawing a typical leaf shape with a black marker onto a piece of clear perspex lying over the top of the leaves on

the light table. The perspex also held the leaves flat to enable a more acurate measurement of leaf area. Two levels of extrapolated leaf area were taken. Firstly, the leaf area loss caused by chrysomelid damage followed by an estimated whole leaf area. Both estimates were conservative estimates when the extrapolation proved difficult or indefinite. Chrysomelid damage is quite characteristic, causing the scalloping of the leaf edges (Greaves 1966, Kile 1974), so the extrapolated leaf area after damage from these insects would therefore be more reliable than total leaf area loss since area loss was often more predictable.

Data analysis

All data were checked for a normal distribution of individual tree residuals. Those traits with normal distributions were analysed using the GLM procedure in SAS (1992). Those data that were not normal were analysed using the CATMOD procedure in (SAS 1992). Both methods are outlined below.

GLM analysis

The percentage of the total leaf area lost from chrysomelid damage and total leaf area lost were normal after transformation ($X^{0.25}$). These traits were analysed using analysis of variance and the GLM procedure of SAS (1992). The significance of cross type means (*E. nitens* [N], *E. globulus* [G] or *E. nitens x E. globulus* hybrids [H]), was initially investigated using model [1]. However, as cross was not found to be significant (see results), the data were further investigated using model [2].

[1] y = cross type + position + age + tree(cross) + position x age +cross x position + cross x age + cross x position x age + age x tree(cross)
+ position x tree(cross) + position x age x tree(cross) + error

where position was equal to the position (top or bottom of the canopy) where the branchlet sample was taken, age was the age (new season's or old season's) of the leaf sample taken and, error was the residual error. Cross type, position and age were all treated as fixed effects.

[2] y = family + position + age + tree(family) + family x position + family x age
+ position x age + family x position x age + age x tree(family) +
position x tree(family) + position x age x tree(family) + error

where family, position and age were all treated as fixed effects.

Arithmetic means were determined for the cross type effects. Differences between individual crosses or individual families were determined using Tukey's studentised range test and type III sum of squares.

CATMOD analysis

Traits that had residuals significantly different from normal were analysed using the CATMOD procedure of SAS (1992). These traits included the %loss (other), %new nodes, % old nodes, laterals (see Table 4.3). All traits were divided into categories (given below), and the number of individual scores within the classes determined. Analysis of these classes was then undertaken in CATMOD, using cumulative logits for ordinal data. Cross type and the response function were included in the model. Contrasts between cross types were undertaken using the contrast option, and probabilities obtained from Chi-square values based on the Wald statistic.

The categories used for analysis of %loss (other) were no leaf area loss (1); 1-5% leaf area loss (2); >5-10% (3); >10%-20 (4); >20% (5). The number of individuals in each category were determined for each cross type and these data were then used for analysis of this trait, for cross type means, family means and differences between sample position and leaf age. The number of laterals present on each sample from each tree was divided into 8 categories: zero or one lateral per sample (1); two laterals per sample (2); three laterals per sample (3); four laterals per sample (4); five laterals per sample (5); 6, six laterals per sample (6); seven laterals per sample (7); and eight or more laterals per sample (8). The data were first analysed for all values when divided into these eight categories. A second analysis was also run, using data only from samples with one or more laterals (ie. laterals>0). The traits %new nodes and %old nodes were divided into the same 5 categories: 0 to 25% (1); 25 to 50% (2); 50 to 75% (3); 75 to 99% (4) and 100% (5).

Results

Qualitative damage assessment

Chrysomelid damage on *E. nitens x E. gunnii* hybrids was consistently higher than damage on *E. gunnii* at both early (P<0.000) and late (P=0.027) scorings of chrysomelid damage (Figure 4.1, Table 4.4, 4.5). The *E. nitens x E. gunnii* hybrids also had significantly higher damage than *E. nitens* early in the season (P<0.000), however the difference was no longer significant later in the season (P= 0.144). Mean damage on *E. nitens x E. globulus* hybrids was consistently higher than the pure parent species, *E. nitens* or *E. globulus*, although these differences were not significant (Figure 4.1, Table 4.4, 4.5).

The majority of F_1 hybrids had greater chrysomelid damage than the maternal parent *E. nitens*, and damage greater than or equal to the most damaged parent, although this was not often significant (Table 4.4, 4.5). The F_1 hybrids between *E. nitens* and *E. viminalis* and *E. nitens* and *E. pulverulenta* were consistently among the most highly damaged cross types in the trial. Chrysomelid damage was found to be significantly different between cross types when scored both relatively early in the season (February 1993, Figure 4.1 i, Chi-square P<0.000) and late in the season (April 1993, Figure 4.1 ii, Chi-square P<0.003). Early in the season, there were no significant differences between any pure species controls. However, the trend in damage levels for species with several families respresented was: *E. globulus* ² *E. gunnii* ² *E. nitens* (Figure 4.1).

One of the most notable difference between the trends in the early and late scorings of chrysomelid damage was the relative position of CAM (*E. camaldulensis*). Early in the season, it had low damage levels, but by April it has the highest damage level in the trial (Figure 4.1).

Damage caused by *Uraba lugens* was low on all cross types, but was significantly different between cross types (Chi-square P<0.000). The *E. nitens x E. gunnii* F_1 hybrids were significantly more damaged by *U. lugens* than both pure parent species (*E. nitens* or *E. gunnii*, Figure 4.1 iii, Table 4.6). Similarly, the *E. nitens x E. globulus* F_1 hybrids were significantly more damaged than *E. nitens*, although not significantly more damaged than *E. globulus*. In the majority of cases, the F_1 hybrids were more damaged or at least as damaged as both the maternal parent (*E. nitens*) and the paternal parent. However, there were instances where the hybrid had a smaller mean damage than both parents (*E. nitens x E. camaldulensis*) or at least one parent species (*E. nitens x E. globulus*, *E. nitens x E. cordata*) although this difference was not significant.

Damage caused by *Uraba lugens* within the trial showed the following basic trend, for pure species with more than one family constituting the cross type:

E. globulus > *E. nitens* = *E. gunnii*

(See Table 4.6 for probabilities). Certainly, the most susceptible species were *E. globulus*, *E. cordata* and *E. johnstonii*.

Quantitative leaf area loss

Leaf area loss

Total leaf area loss did not differ significantly between cross types G, H and N (P=0.508, see Table 4.7). However, the trend in leaf area loss indicated that damage

caused by chrysomelids was greatest on *E. globulus*, *E. nitens* had the least damage and the hybrids were intermediate (Figure 4.2 i). % loss (other) was greatest on the F_1 hybrid and *E. nitens* when compared with *E. globulus* (Figure 4.2 ii). Overall, this translated to total damage levels (%loss) being highest on the hybrids, then *E. nitens* and the least on *E. globulus*. This trend was also observed on *E. nitens*, *E. globulus* and their F_1 hybrid in the qualitative assessments.

When cross type classification was ignored and differences between families were examined, significant family effects were determined for %loss (chrysomelid) and %loss (other), ($P^2 0.005$ for both, Table 4.8 for chrysomelids, significance of family effect in CATMOD analysis of %loss (other)). % loss was not found to differ significantly between families (P= 0.076). Significant position effects were detected for both % loss and % loss (chrysomelids) (Table 4.8). However, differences in the sample age was significant only for total % loss and %loss (chrysomelids) (where cross was included in the model, Tables 4.7 and 4.8). Significant variation at the tree within family level (tree[family], Table 4.8) for total % loss implied that there was considerable variation between trees within a family. Similarly, there was significant variation in the interactions which contained the family within tree term for total % loss which suggested that this variation extended to the age and position levels in the model.

At the family level, *E. globulus* generally had the highest value for % loss (chrysomelids) (Figure 4.3). In one instance, *E. nitens x E. globulus* hybrids were found to have significantly less damage than the *E. globulus* parental control (H54 vs G500, Figure 4.3). There was significant variation within the *E. globulus* families, with G500 having significantly more % loss (chrysomelids) than G176 (Figure 4.3). In most cases (4 out of 5) *E. globulus* and *E. nitens* did not differ significantly in % loss (other) (see Figure 4.4). However, in one instance the *E. nitens* family had significantly greater % loss (other) than the *E. globulus* control (G500 vs. N597). Hybrids were found to be either significantly more damaged (% loss [other]) than both parental control families (3 out of 5 cases) or intermediate between the parents (2 out of 5 cases). At the family level, there was very little difference between % loss although a significant difference between *E. globulus* families was again determined (G158 vs. G176, Figure 4.5). In only one instance (H3 vs G176), the F_1 hybrid family was found to have a higher % loss than one of its pure parent species.

Sample age and position

Percentage loss (chrysomelids) was found to be significantly greater on the samples taken from the top of the tree canopy than samples taken from the bottom of the

canopy (Figure 4.2 ii). However, % loss (other) was found to be significantly greater on the samples taken from the bottom of the canopy (Figure 4.2 ii). Overall, total percentage loss was greatest on samples taken from the top of the canopy when compared with samples taken from the bottom of the canopy (Figure 4.2 ii). New seasons growth always had greater leaf area loss than old season's growth (Figure 4.2 iii). This difference was significant for total percentage loss and % loss (other), although it was not significant for % loss (chrysomelids) (see Figure 4.2).

Whole leaf loss

The percentage of new nodes occupied by leaves was found to be significantly lower for the *E. nitens* and *E. globulus* F_1 hybrids when compared with their parental controls (see Figure 4.6). In fact, the hybrids had less than 50% of the new season's nodes occupied by leaves. *E. globulus* had significantly greater percentage old nodes occupied by leaves than either hybrids or *E. nitens* (see Table 4.10). However for old season's growth, *E. globulus* had more than 35% nodes occupied and *E. nitens* and the hybrids had less than 25% nodes occupied.

Laterals

The number of laterals present on each sample from each tree was found to be not significantly different between cross types (see Table 4.11, Figure 4.7 i). However, if the analysis was restricted to those samples which had any laterals, then *E. nitens* was found to have significantly more laterals than any other cross type (Figure 4.7 ii, Table 4.12). Again, the F_1 hybrids had the lowest number of laterals (if laterals were present), although not significantly lower than *E. globulus*.

Discussion

Damage caused by the gum leaf skeletoniser, *Uraba lugens* occurred preferentially on *E. globulus* and *E. nitens* x *E. globulus* F_1 hybrids. Overall, damage levels were very low throughout the trial and it is unlikely that this insect would cause significant loss of growth in any of the cross types examined, even on the most damaged cross type *E. globulus*. This is consistent with the fact that *Uraba lugens* is largely classified as a low level pest in eucalypt forests (Howes 1990). However, outbreaks have been shown to occur in natural forests (Campbell 1962) and more resistant species such as *E. nitens* should perhaps be considered for plantations in areas that favour this pest. *Uraba lugens* damage levels on the hybrids between eucalypt species were inherited predominantly in an additive manner. However, levels on the hybrids were often closer to the damage levels determined for the most

damaged parent and *E. nitens x E. gunnii* hybrids were more damaged by *U. lugens* than both parent species

The amount of qualitative damage levels caused by chrysomelids on the different cross types differed between early season and late season scorings of the same field trial. Late season scorings were generally higher than early season's scorings and F_1 hybrids were consistently among the most damaged cross types in both instances. The least damaged species were *E. pulverulenta, E. cordata, E. globulus* and *E. nitens*.

Qualitative chrysomelid damage was in general greater on the F_1 hybrids than either pure parent control or the hybrids were at least as damaged as the most damaged parent. *E. nitens x E. gunnii* hybrids were more damaged by chrysomelids than both parent host species. In general, hybrids were rarely significantly more damaged than both parents, although mean damage levels on the hybrids were often higher. Hence, the inheritance of damage levels on the hybrids generally followed the dominance hypothesis of (Fritz *et al.* 1994), where hybrids differed significantly from the mean resistance of both parents, but not from one of the parents. However, *E. nitens x E. gunnii* hybrids were more susceptible than parent species and it is possible that with more replication, other hybrids may have been more susceptible than parent species.

A total leaf area loss of around 12 to 13% was determined for *E. globulus* , *E. nitens* and their F_1 hybrid. This leaf area loss is comparable with the estimates of 15% obtained by (Fox and Morrow 1983) and 10% (maximum) by Ohmart *et al.* (1983). This estimate is lower than overall estimates of damage in Australia, of around 15 to 50%. Certainly, it is well below the estimated defoliation of 40% by Kile (1974). However, the chrysomelid damage was not severe on these cross types and these estimates do not include the leaf area loss from unoccupied nodes. Loss of whole leaves are not often included in leaf area loss estimates because of the difficulty of obtaining an accurate estimate of leaves that are completely consumed and in directly attributing the loss of the same leaves to insect damage (Ohmart *et al.* 1984). Furthermore, there were more susceptible cross types present within the immediate vicinity (eg. *E. camaldulensis*) and this may have contributed to the lower percentage leaf area loss since, in natural forests there is a lower number of host choices.

Total leaf area loss was greatest on the new season's foliage and in the top half of the canopy. This was not unexpected, since this is the most actively growing portion of the canopy and would therefore most likely have the most consistently available new

shoots as a food source. Strauss and Morrow (1988) determined that one of the best predictors of beetle numbers was the amount of new foliage, and hence the number of growing tips, available at any one time. No difference between cross types in the number of laterals on each sample taken was determined in this experiment. It seems unlikely that the number of growing points or the number of laterals is affecting host choice, although when laterals were present, *E. nitens* had the greatest number of laterals.

E. globulus had the lowest mean total percentage leaf area loss (although not significant, see Figure 4.2, 4.6), as well as having a high percentage of leaves retained in both new and old season's growth. This suggests that perhaps one of *E. globulus*'s growth strategies is to retain maximum leaf area for faster growth. *E. nitens* however, had low percentage nodes occupied by leaves for old season's growth which in turn suggests that perhaps high leaf area is not maintained by keeping older foliage intact in this species.

Mean total leaf area loss on hybrids between *E. nitens* and *E. globulus*, was higher than both *E. nitens* and *E. globulus* although this difference was not significant. At a family level, the hybrids had variable responses but were generally at least as susceptible at the most damaged parents, following the dominance hypothesis of Fritz *et al.* (1994). However, both hybrid susceptibility (percentage leaf area loss from other causes); and hybrid resistance (for chrysomelid damage) responses were found.

It was clear from these experiments that, in terms of chrysomelid damage, *E. nitens* and *E. globulus* are probably good choices for plantation species in chrysomelid prone areas. The relatively low amount of damage that these cross types had from the qualitative data indicated, at least in a multi-species situation that these species were among the most resistant. Hybrids between species were generally at least as damaged as the most damaged pure species control. However, since a case of hybrid resistance was found at the family level, it is possible that the hybrids may be taken advantage of in the future by examining variation at the family level.

Table 4.1. Subjective scoring system used to evaluate each individual tree in the hybrid trial for damage caused by chrysomelid beetles and by the gum leaf skeletoniser, *Uraba lugens*.

score	description of chrysomelid damage	description of *Uraba lugens* damage
1	very little or no damage, canopy outline complete	very little or no damage
2	light defoliation, canopy still in reasonable order, but leaf area loss clearly visible	damage immediately visible, but light defoliation/leaves skeletonised
3	canopy thinned, most of new growth affected but not greater than 50%	damage immediately visible, number of leaves skeletonised moderate but not greater than 50%
4	canopy thinned, new growth damaged but most of leaves still have >50% of leaf area	greater than 50% of foliage skeletonised. Canopy affected but still largely intact
5	new season's growth severely damaged but some leaf area still present	canopy thinned, a large proportion of leaves affected but some new season's foliage intact
6	new season's growth almost completely or completely stripped. Bare branch ends visible over the whole canopy	canopy severely affected, new season's foliage all damaged or nearly all damaged. Bare branches clearly visible.

Table 4.2. Crossing design of families sub-sampled from the *Eucalyptus* hybrid trial. Controls for *E. globulus* were open pollinated progeny whereas controls for *E. nitens* were intra-specific outcrosses.

		***E. globulus* males**		
		G500	**G158**	**G176**
***E. nitens* females**	**N599**	H53		
	N601	H54	H56	
	N597	H48		
	N528			H3

Table 4.3. Traits scored and traits derived from the 40cm branchlet samples taken. Trait names assigned to these are used subsequently in the text. * Note, % leaf area losses were calculated for each leaf age separately but because age was accounted for in the model, the final traits were known only as % loss, % loss (chrysomelids) and % loss (other).

traits scored	trait name	traits derived	derived trait name
•number of nodes along main stem	no. nodes	•% new season's nodes occupied by leaves	%new nodes
•no. leaves on main stem		•% nodes old season's growth occupied by leaves	%old nodes
•node at which new season's foliage begins			
•no. laterals per sample	laterals		
•for new season's growth on main stem:		•Combined *	
•leaf area	%loss new	•% leaf area loss	%loss
•leaf area extrapolated for chrysomelid damage	%loss new (chryosmelids)	•% leaf area loss due to chrysomelids	%loss (chryosmelids)
•extrapolated leaf area	%loss new (other)	•% leaf area loss due to other factors (other insects and mechanical damage)	%loss (other)
•for old season's growth on main stem:			
•leaf area	%loss old		
•leaf area extrapolated for chrysomelid damage	%loss old (chryosmelids)		
•extrapolated leaf area	%loss old (other)		

Table 4.4. Pairwise comparisons for different cross type means for chrysomelid damage scored qualitatively early in the season (February 1993; 1 to 6). Significant differences are given in bold. All probabilities were obtained from specific contrasts using the CATMOD procedure in SAS (see methods).

	CAM	COR	GLO	GUN	JOH	MOR	PUL	ROD	NIT	XCAM	XCOR	XGLO	XGUN	XJOH	XMOR	XOVA	XPUL	XROD
COR	0.561																	
GLO	0.778	0.631																
GUN	0.677	0.695	0.779															
JOH	0.971	0.480	0.632	0.471														
MOR	0.354	0.146	0.094	0.054	0.256													
PUL	0.962	0.966	0.963	0.964	0.962	0.957												
ROD	0.417	0.215	0.250	0.208	0.378	0.881	0.956											
NIT	0.763	0.621	0.992	0.694	0.586	0.071	0.963	0.237										
XCAM	0.109	**0.037**	**0.000**	**0.000**	**0.021**	0.548	0.955	0.852	**0.000**									
XCOR	0.470	0.197	0.135	0.074	0.364	0.768	0.958	0.729	0.099	0.285								
XGLO	0.799	0.343	0.253	0.080	0.744	0.285	0.961	0.434	0.126	**0.004**	0.421							
XGUN	0.184	0.063	**0.000**	**0.000**	**0.044**	0.832	0.956	0.961	**0.000**	0.419	0.503	**0.006**						
XJOH	0.758	0.702	0.926	0.943	0.651	0.141	0.964	0.263	0.924	**0.009**	0.201	0.401	**0.018**					
XMOR	0.978	0.542	0.748	0.646	0.997	0.360	0.962	0.427	0.731	0.104	0.482	0.825	0.182	0.731				
XOVA	0.207	0.094	0.088	0.067	0.163	0.543	0.953	0.717	0.080	0.757	0.407	0.184	0.571	0.103	0.212			
XPUL	0.055	**0.018**	**0.000**	**0.000**	**0.008**	0.306	0.953	0.646	**0.000**	0.485	0.133	**0.001**	0.146	**0.003**	0.051	0.989		
XROD	0.858	0.654	0.949	0.838	0.793	0.232	0.963	0.326	0.942	**0.043**	0.320	0.590	0.081	0.904	0.834	0.142	**0.019**	
XVIM	**0.031**	**0.011**	**0.002**	**0.001**	**0.010**	0.153	0.949	0.378	**0.001**	0.225	0.075	**0.007**	0.103	**0.005**	**0.032**	0.633	0.443	**0.014**

NOTE: PUL = *E. pulverulenta*, CAM= *E. camaldulensis*, GUN = *E. gunnii;* XJOH= *E. nitens x E. johnstonii;* GLO= *E. globulus;* XROD= *E. nitens x E. rodwayi;* COR= *E. cordata;* NIT =*E. nitens;* JOH = *E. johnstonii;* XGLO= *E. globulus;* ROD= *E. rodwayi ;* XOVA= *E. nitens x E. ovata;* MOR= *E. morisbyi;* XGUN= *E. nitens x E. gunnii;* VIM= *E. viminalis;* XVIM= *E. nitens x E. viminalis;* XCAM= *E. nitens x E. camaldulensis;* XMOR= *E. nitens x E. morrisbyi;* XPUL= *E. nitens x E. pulverulenta.*

Table 4.5. Pairwise comparisons for different cross type means for chrysomelid damage scored qualitatively in April 1993 (1 to 6). Significant differences are given in bold. All probabilities were obtained from specific contrasts using the CATMOD procedure in SAS (see methods). See Table 4.4 for cross type codes.

	CAM	COR	GLO	GUN	JOH	MOR	PUL	ROD	NIT	XCAM	XCOR	XGLO	XGUN	XJOH	XMOR	XOVA	XPUL	XROD
COR	**0.003**																	
GLO	**0.000**	0.638																
GUN	**0.000**	0.828	0.474															
JOH	**0.004**	0.365	0.385	0.133														
MOR	**0.002**	0.765	0.864	0.830	0.448													
PUL	0.908	0.928	0.926	0.927	0.923	0.926												
ROD	**0.011**	0.744	0.980	0.814	0.637	0.933	0.926											
NIT	**0.004**	0.222	**0.048**	**0.000**	0.650	0.222	0.922	0.460										
XCAM	**0.002**	0.366	0.312	**0.040**	0.909	0.443	0.923	0.659	0.380									
XCOR	**0.000**	0.959	0.419	0.657	0.167	0.645	0.928	0.658	**0.038**	0.133								
XGLO	**0.001**	0.419	0.439	0.088	0.782	0.525	0.924	0.725	0.270	0.823	0.180							
XGUN	**0.001**	0.407	0.369	**0.027**	0.761	0.505	0.924	0.719	0.144	0.787	0.153	0.998						
XJOH	**0.004**	0.484	0.639	0.342	0.801	0.622	0.924	0.772	0.490	0.846	0.301	0.960	0.959					
XMOR	**0.001**	0.644	0.986	0.558	0.445	0.863	0.925	0.974	0.123	0.409	0.449	0.527	0.484	0.677				
XOVA	**0.009**	0.480	0.637	0.398	0.897	0.611	0.924	0.743	0.655	0.949	0.329	0.959	0.957	0.938	0.664			
XPUL	**0.031**	0.087	0.011	**0.000**	0.186	0.066	0.919	0.213	0.178	0.071	**0.009**	0.053	**0.030**	0.151	**0.027**	0.265		
XROD	**0.001**	0.819	0.410	0.572	0.206	0.559	0.929	0.572	0.098	0.195	0.819	0.233	0.220	0.305	0.424	0.316	**0.032**	
XVIM	**0.001**	0.580	0.843	0.420	0.535	0.769	0.925	0.903	0.172	0.515	0.370	0.646	0.611	0.775	0.874	0.745	**0.038**	0.368

Table 4.6. Pairwise comparisons for different cross type means for gum leaf skeletoniser (*Uraba lugens*) damage scored qualitatively in April 1993 (1 to 6). Significant differences are given in bold. All probabilities were obtained from specific contrasts using the CATMOD procedure in SAS (see methods). See Table 4.4 for cross type codes.

	CAM	COR	GLO	GUN	JOH	MOR	PUL	ROD	NIT	XCAM	XCOR	XGLO	XGUN	XJOH	XMOR	XOVA	XPUL	XROD
COR	0.414																	
GLO	0.283	0.947																
GUN	0.205	**0.009**	**0.000**															
JOH	0.602	0.613	0.331	**0.003**														
MOR	0.849	0.301	0.176	0.298	0.440													
PUL	0.958	0.952	0.952	0.964	0.955	0.959												
ROD	0.955	0.950	0.950	0.962	0.953	0.957	0.998											
NIT	0.632	0.070	**0.000**	0.089	**0.047**	0.823	0.960	0.958										
XCAM	0.512	0.062	**0.001**	0.309	0.056	0.681	0.961	0.959	0.680									
XCOR	0.893	0.371	0.140	**0.038**	0.578	0.712	0.957	0.955	0.309	0.250								
XGLO	0.299	0.922	0.891	**0.000**	0.356	0.187	0.953	0.950	**0.000**	**0.001**	0.149							
XGUN	0.734	0.399	**0.021**	**0.001**	0.655	0.541	0.956	0.954	**0.009**	**0.041**	0.760	**0.018**						
XJOH	0.500	0.769	0.573	**0.003**	0.792	0.357	0.954	0.952	**0.036**	**0.041**	0.445	0.616	0.468					
XMOR	0.801	0.176	**0.026**	0.131	0.241	0.983	0.959	0.957	0.744	0.566	0.584	**0.026**	0.289	0.178				
XOVA	0.421	0.930	0.835	**0.004**	0.634	0.298	0.953	0.951	**0.039**	**0.040**	0.360	0.886	0.369	0.819	0.149			
XPUL	0.912	0.332	0.086	**0.027**	0.514	0.724	0.957	0.955	0.275	0.229	0.966	0.089	0.682	0.386	0.585	0.312		
XROD	0.919	0.350	0.222	0.251	0.512	0.929	0.958	0.956	0.732	0.602	0.795	0.235	0.629	0.420	0.897	0.352	0.811	
XVIM	0.878	0.343	0.077	**0.017**	0.536	0.687	0.957	0.955	0.203	0.181	0.987	0.078	0.722	0.399	0.527	0.321	0.947	0.775

Table 4.7. ANOVA table for chrysomelid damage where cross type was included in the model (GLM, based on type III sums of squares).

		% loss		% loss (chrysomelids)	
Source	df	F value	Pr > F	F value	Pr > F
cross	2	0.68	0.508	2.04	0.135
position	1	15.41	0.000	69.43	0.000
age	1	19.46	0.000	24.83	0.000
posn*age	1	0.13	0.714	2.21	0.138
cross*posn	2	7.64	0.001	3.30	0.374
cross*age	2	3.40	0.034	6.01	0.003
cross*posn*age	2	6.72	0.001	3.67	0.026
tree(cross)	55	2.38	0.000	6.17	0.000
age*tree(cross)	55	2.01	0.000	2.27	0.000
posn*tree(cross)	55	1.57	0.007	2.71	0.000
posn*age*tree(cross)	28	1.56	0.035	1.99	0.002

What are fixed etc.
don't know what is tested against what

Table 4.8. ANOVA table for %loss and %loss (chrysomelids) where family instead of cross type was included in the model (GLM, based on type III sums of squares).

		% loss		% loss (chrysomelids)	
Source	df	F value	Pr > F	F value	Pr > F
family	11	1.67	0.076	2.800	0.005
position	1	15.37	0.000	15.44	0.000
age	1	15.31	0.000	0.174	0.678
posn*age	1	0.04	0.844	0.061	0.807
family*posn	11	1.77	0.056	0.634	0.791
family*age	11	3.47	0.000	0.700	0.733
family*posn*age	11	1.78	0.054	0.246	0.989
tree(family)	46	2.22	0.000	1.170	0.470
age*tree(family)	46	1.58	0.011	0.804	0.727
posn*tree(family)	46	1.78	0.002	1.263	0.330
posn*age*tree(family)	19	1.96	0.008	1.934	0.010

Table 4.9. Table of contrasts between cross types for the percentage of new seasons nodes occupied by leaves (% new nodes; see Table 4.3) Note: N is *E. nitens*, G is *E. globulus* and H is *E. nitens x E. globulus* F_1 hybrids.

contrast	d.f.	chi-squared	P
cross	2	9.39	0.009
N vs G	1	1.14	0.593
N vs H	1	1.14	0.006
G vs H	1	0.00	0.008

Table 4.10. Table of contrasts between cross types for the percentage of old seasons nodes occupied by leaves (% old nodes; see Table 4.3). Note: N is *E. nitens*, G is *E. globulus* and H is *E. nitens x E. globulus* F_1 hybrids.

contrast	d.f.	chi-squared	P
cross	2	55.97	0.000
N vs G	1	54.66	0.000
N vs H	1	1.58	0.209
G vs H	1	30.67	0.000

Table 4.11. Table of contrasts for differences between cross types for all observations on the number of laterals per sample. Note: N is *E. nitens,* G is *E. globulus* and H is *E. nitens x E. globulus* F_1 hybrids.

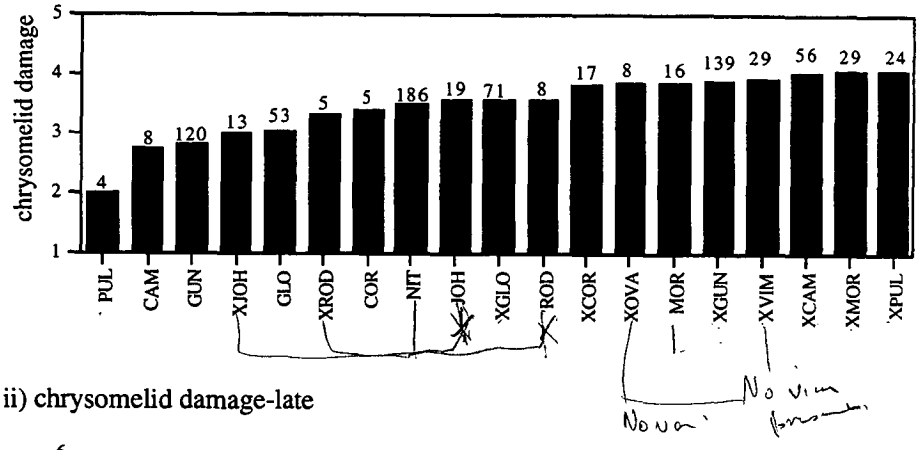
contrast	d.f.	chi-squared	P
cross	2	1.42	0.492
N vs G	1	1.14	0.2854
N vs H	1	1.14	0.2854
G vs H	1	0.00	1.0000

Table 4.12. Table of contrasts between cross types for observations greater than zero, on the number of laterals per sample. Note: N is *E. nitens,* G is *E. globulus* and H is *E. nitens x E. globulus* F_1 hybrids.

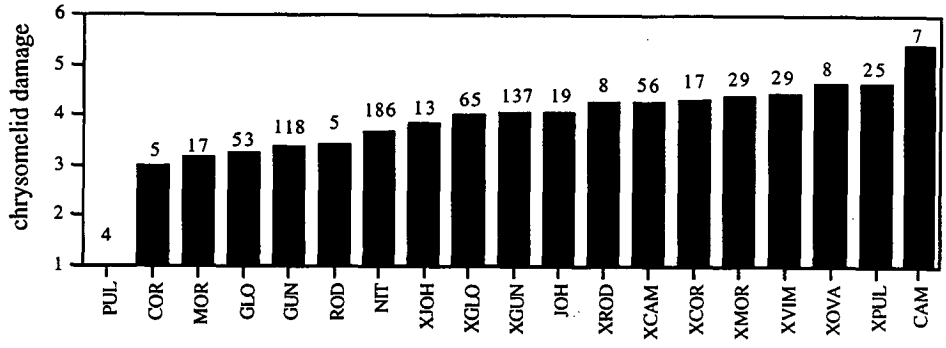
contrast	d.f.	chi-squared	P
cross	2	14.82	0.001
N vs G	1	12.03	0.001
N vs H	1	12.03	0.001
G vs H	1	0.00	1.000

Figure 4.1. Cross type means of chrysomelid damage scored qualitatively i) early in the summer season, February 1993; ii) late in the season, April 1993 (1 to 6) and; iii) damage by *U. lugens* scored in April 1993. Numbers above cross types indicate the number of individuals in each cross that were assessed. Pairwise comparisons of these cross types are given in Table 4.4, 4.5 and 4.6.

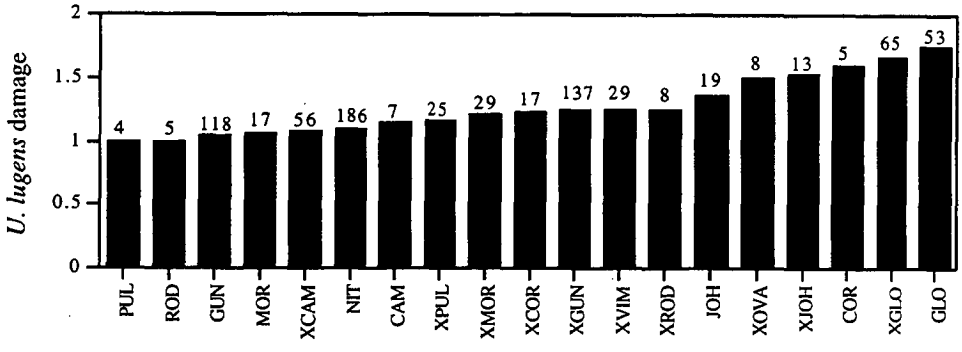
i) chrysomelid damage-early



ii) chrysomelid damage-late



iii) *U. lugens* damage-late



Note: PUL = *E. pulverulenta*, CAM= *E. camaldulensis;* GUN = *E. gunnii;* XJOH= *E. nitens x E. johnstonii;* GLO= *E. globulus;* XROD= *E. nitens x E. rodwayi;* COR= *E. cordata;* NIT =*E. nitens;* JOH = *E. johnstonii;* XGLO= *E. globulus;* ROD= *E. rodwayi ;* XOVA= *E. nitens x E. ovata;* MOR= *E. morisbyi;* XGUN= *E. nitens x E. gunnii;* VIM= *E. viminalis;* XVIM= *E. nitens x E. viminalis;* XCAM= *E. nitens x E. camaldulensis;* XMOR= *E. nitens x E. morrisbyi;* XPUL= *E. nitens x E. pulverulenta.*

Figure 4.2. Means of leaf area loss for i) cross types, ii) sample position and iii) leaf age, assessed quantitatively for % loss (chrysomelids), % loss (other) and % loss. Note: G = *E. globulus,* N = *E. nitens,* H= *E. nitens x E. globulus* hybrids. Differences between cross types were not found to be significant in any case (see Table 4.7). Different letters above the columns represents a significant difference where P≤0.05. For % loss (chrysomelids) and % loss, this difference was obtained using Tukey's studentized range test. For % loss (other), these differences were obtained from Chi-square pairwise contrasts using CATMOD (see methods).

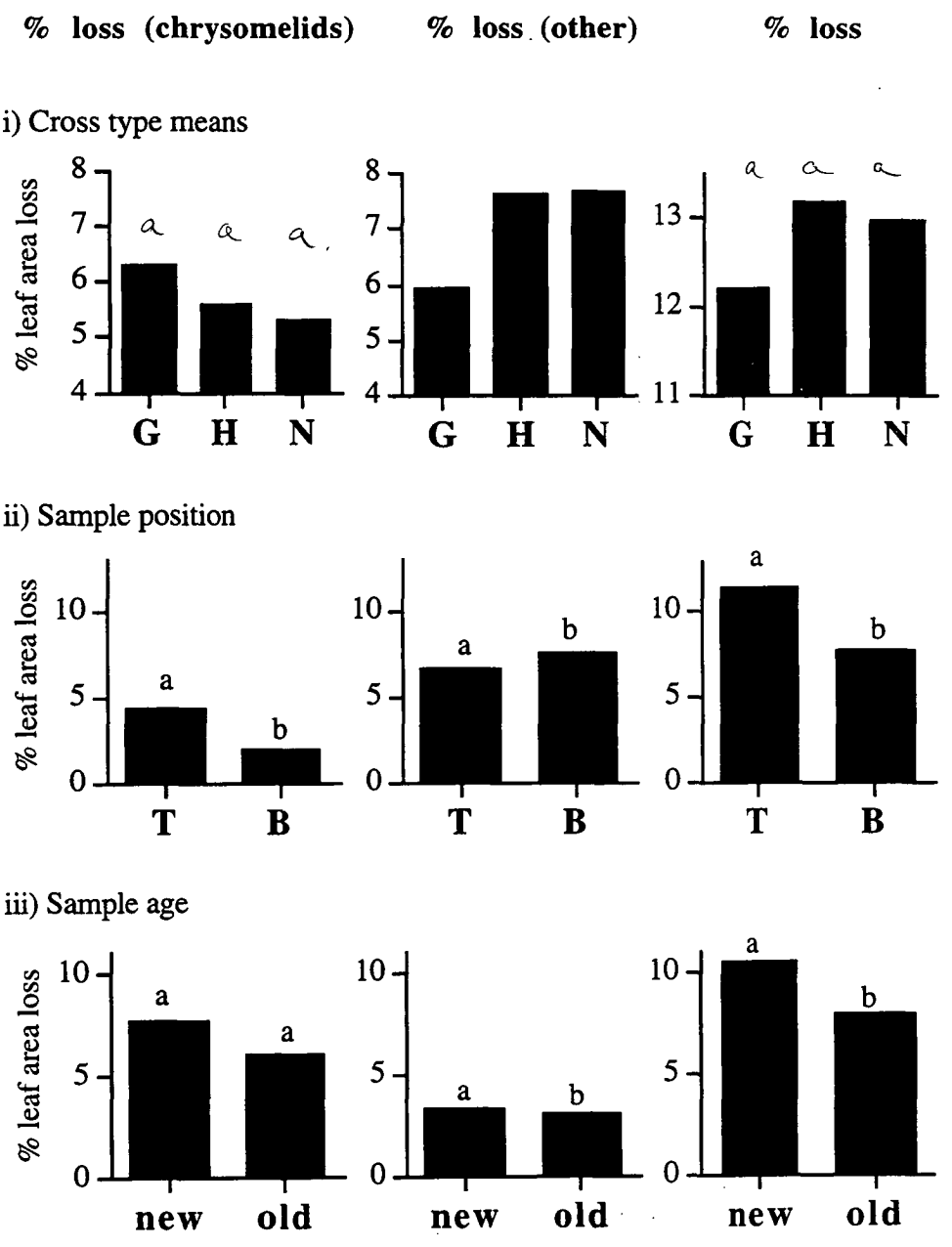


Figure 4.3. Family means for the % loss (chrysomelids). Families are grouped into a hybrid family (H) and its respective parental controls (G and H). Significant differences, obtained using Tukey's studentized range test (for all families), at the 0.05 level are represented by different letters above the families.

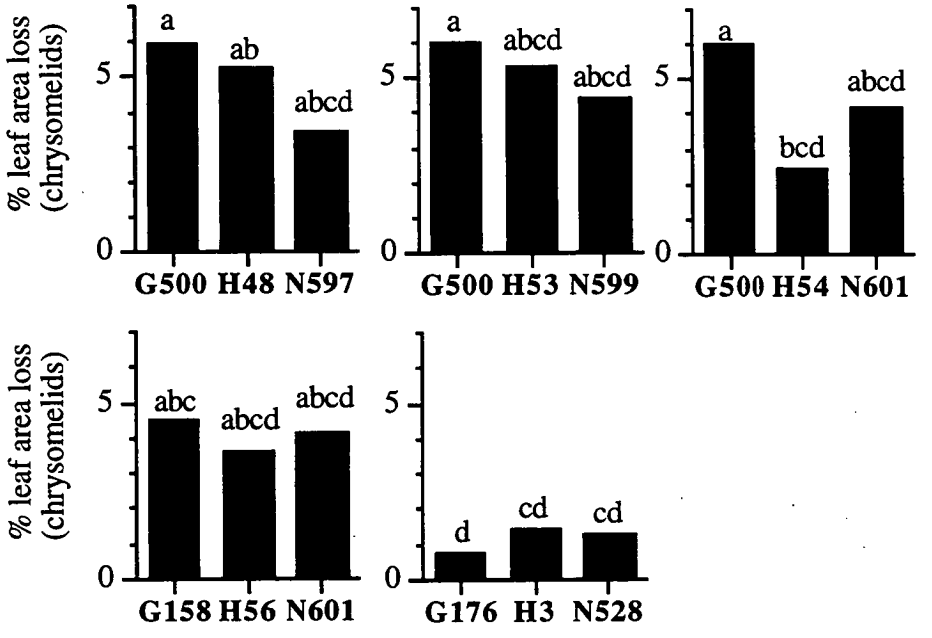


Figure 4.4. Family means for the % loss (other). Families are grouped into a hybrid family (H) and its respective parental controls (G and H). Significant differences, obtained using the CATMOD procedure of SAS (for all families, see methods), at the 0.05 level are represented by different letters above the families.

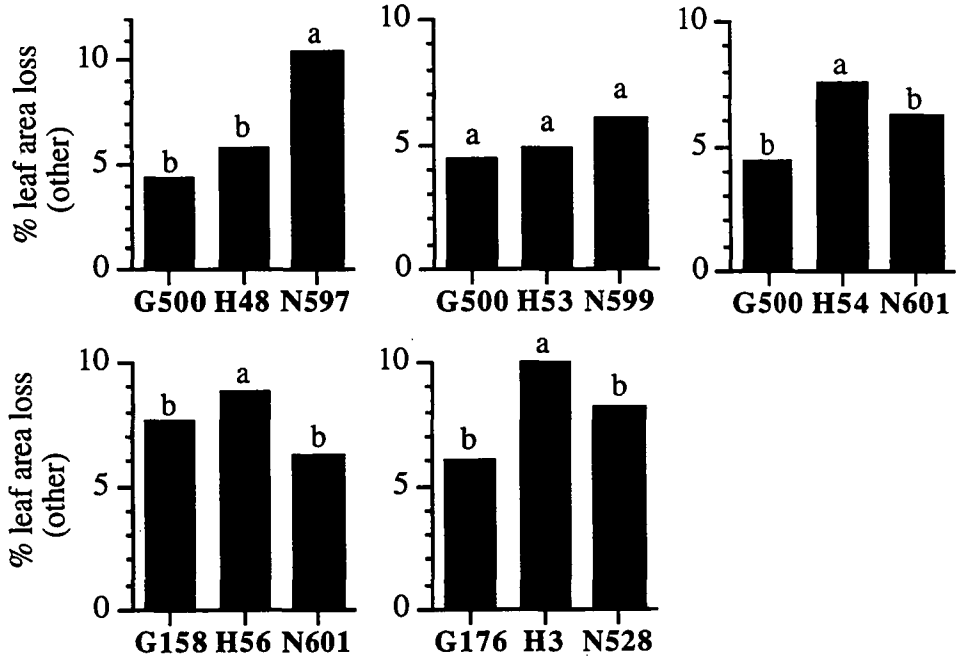


Figure 4.5. Family means for percent of total leaf area lost. Families are grouped into a hybrid family (H) and its respective parental controls (G and H). Significant differences, obtained using Tukey's studentized range test (for all families), at the 0.05 level are represented by different letters above the families.

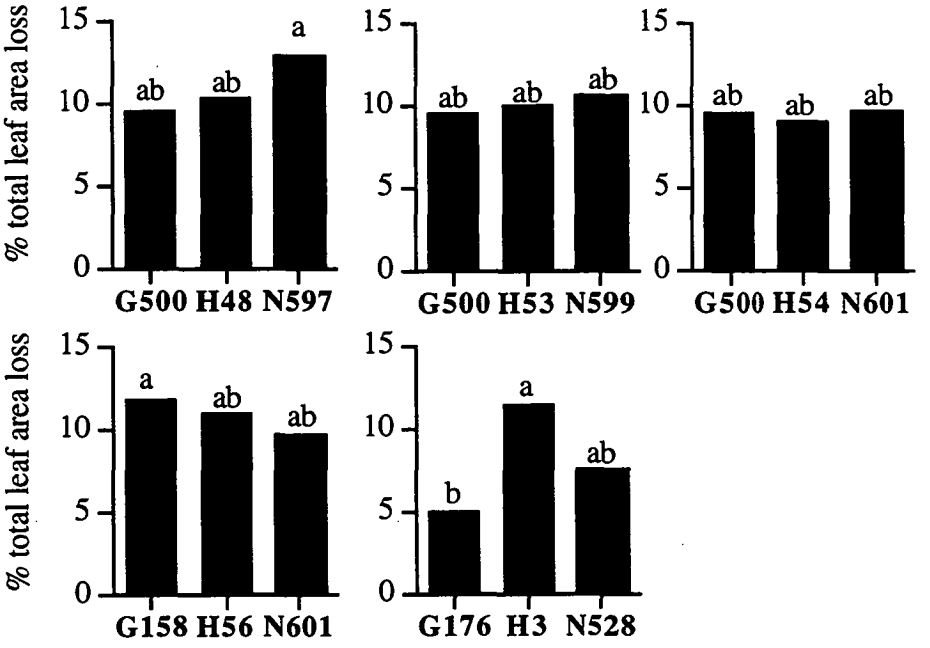


Figure 4.6. Percentage nodes occupied by leaves for i) new and ii) old seasons growth. Different letters above the crosstypes represent significant differences at the P≤0.01 level. These were derived from chi-squared results using the CATMOD procedure in SAS (1992), see Tables 4.10 and 4.11. Note: N is *E. nitens*, G is *E. globulus* and H is *E. nitens x E. globulus* F_1 hybrids.

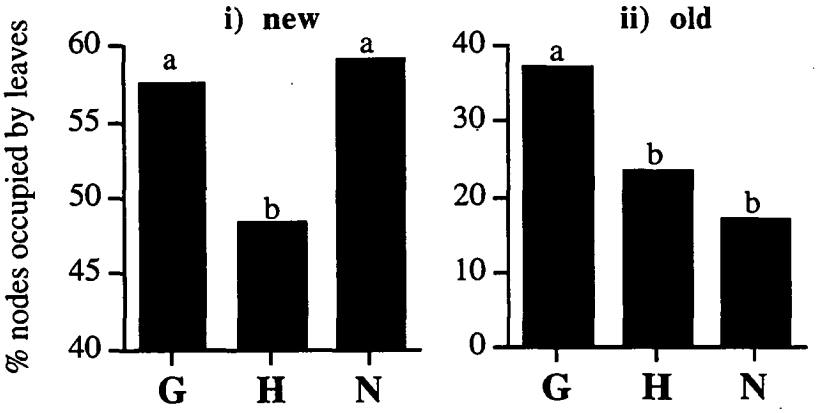
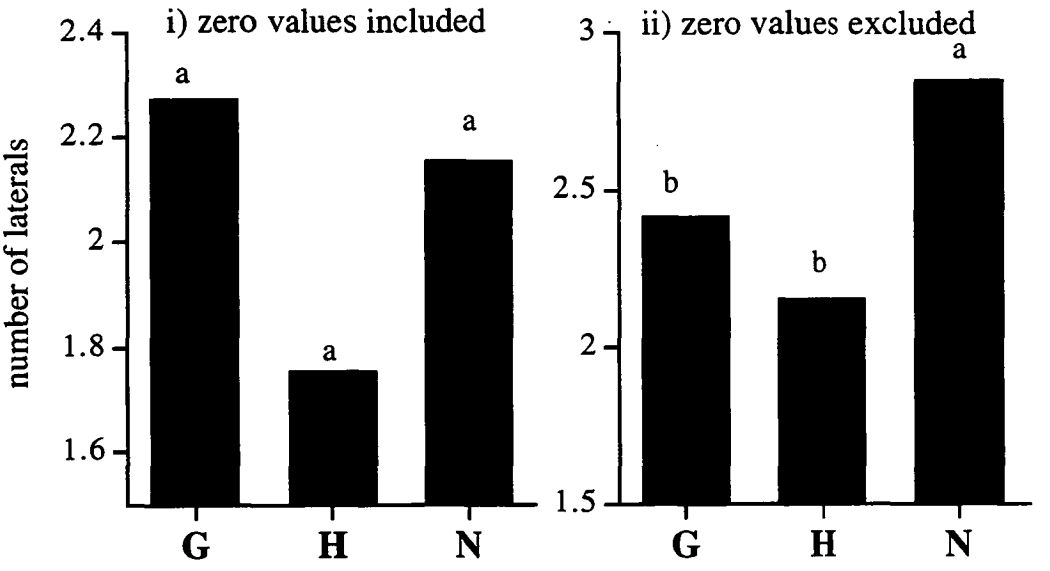


Figure 4.7. The number of laterals per branchlet sample taken, in each cross type for i), where all scores were included, and ii), where samples with no laterals were excluded from the analysis. Different letters above the crosstypes represent significant differences at the P<0.001 level. These were derived from chi-squared results using the CATMOD procedure in SAS (1992), see Tables 4.11 and 4.12. Note: N is *E. nitens,* G is *E. globulus* and H is *E. nitens x E. globulus* F_1 hybrids.



Chapter 5

Plant hybrid zones as centres of biodiversity: evidence from *Eucalyptus amygdalina x E. risdonii* hybrids

Introduction

Whitham (1989) initially proposed that plant hybrids and hybrid zones may act as 'sinks' for pests following a detailed study of a gall producing aphid in a *Populus* hybrid zone. The generality of this response has subsequently been tested in a number of studies as it has important implications for the utilisation of hybrid plants in agriculture and forestry and in the conservation of biodiversity (Boecklen and Spellenberg 1990, Floate and Whitham 1993, Fritz *et al.* 1994, Morrow *et al.* 1994, Strauss 1994). There is mounting evidence to suggest that natural plant hybrid zones may be centres of insect abundance and species richness (Whitham 1989, Whitham *et al.* 1991a, Whitham *et al.* 1991b, Floate *et al.* 1993, Whitham *et al.* 1994), and this pattern could even extend to higher trophic levels (Martinsen and Whitham 1993).

In *Eucalyptus*, natural hybrids have been shown to support greater insect loads and species richness than pure species phenotypes both within the hybrid zone and from adjacent populations (Morrow *et al.* 1994, Whitham *et al.* 1994). Within a natural hybrid zone between *E. risdonii* (R) and *E. amygdalina* (A) in the Government Hills near Hobart Tasmania, Whitham *et al.* (1994) demonstrated that hybrids supported greater insect loads and species richness than pure species phenotypes. Increased species richness and abundance was also observed on pure *E. risdonii* and *E. amygdalina* (A) phenotypes within the natural hybrid zone between these species compared with adjacent pure populations (Whitham *et al.* 1994). This observation may be explained by one or all of five mechanisms proposed by Whitham *et al.* (1994): insects may overflow from hybrids with high herbivore loads to nearby parental types; the close proximity of the two host species could facilitate the mixing of the separate insect faunas of each of the hosts at the species boundary; increased stress at the host species boundaries may make both parental species inately more susceptible to herbivores; expanded leafing-out phenologies in a hybrid zone due to greater heterogeneity could expand the season that trees can be successfully utilized by herbivores; trees classified as pure phenotypes in the hybrid zone may in fact be complex advanced generation hybrids. The possible cause of hybrid susceptibility *per se* has been postulated by Whitham *et al.* (1994) to be due to either i) increased genetic susceptibility of the hybrids due to the breakup of co-adapted gene complexes or, (ii) increased stress at the distributional limits of species increasing susceptibility to attack.

Attempting to directly attribute observed hybrid susceptibility to genetic susceptibility alone means some of these theories must be addressed. Incorrect classification of hybrids and pure species in the hybrid zone can be addressed using molecular methods (e.g. Paige *et al.* 1990, Paige and Capman 1993). Recent investigations in *E. amygdalina x E. risdonii* natural hybrids using molecular markers (RAPDs) suggests that classification by morphology in this hybrid system is largely correct. However, there were examples of inconsistencies between morphology and RAPD classification (Sale *et al.* submitted). It is important to have hybrids of known genetic status through identification using molecular markers or from fully pedigreed crosses (Fritz *et al.* 1994). Addressing some of the other hypotheses, ultimately depends on comparing the responses of pests to plants of known pedigree and genotype in controlled or common environments (Fritz *et al.* 1994). Only through the removal of stress at natural host species boundaries and or randomisation of genotypes can some of the hypotheses be addressed directly.

The nature of hybrid susceptibility is examined in this chapter using both natural and artificial controlled- cross hybrids between *E. amygdalina* and *E. risdonii*, planted in an experimental field trial. The availability of controlled-cross material is a major advantage as it allowed direct and confident comparisons between hybrids and pure species of known pedigree and allows the question of the genetic nature of hybrid susceptibility to be determined directly (Fritz *et al.* 1994). Species richness in both natural populations and in the field trial is discussed and species preference examined for a number of insect taxa within the field trial. The extent of genetic susceptibility in the hybrids is discussed.

Hybrid susceptibility has been discussed largely only at the genetic level in trees and shrubs (e.g. Fritz *et al.* 1994, Whitham 1989). Mechanisms that may be directly responsible for hybrid susceptibility are largely theoretical, for example the breakdown of co-adapted gene sequences (see Fritz *et al.* 1994). In eucalypts, the mechanisms for susceptibility or resistance are postulated to involve secondary plant compounds, leaf waxes, leaf nitrogen content and leaf toughness (e.g. see Edwards 1982, Edwards *et al.* 1993, Fox and Macauley 1977, Lowman and Box 1983, Morrow *et al.* 1976, Morrow and Fox 1980, Ohmart 1991, Ohmart *et al.* 1984, Ohmart *et al.* 1987, Stone and Bacon 1994, White 1984). Potential mechanisms which may have been responsible for the different species preferences examined in this chapter were also investigated. These included leaf toughness and leaf essential oil composition.

Materials and methods

Controlled-cross hybrid trial

The controlled-cross hybrid trial was established by Dr. Brad Potts, University of Tasmania. All the crosses represented in the trial (except the F_2) were from material in and around the natural hybrid zone at Government Hills, near Risdon (see Figure 6.1). The single F_2 family was of unrelated pedigree and originated from the selfing of a putative F_1 hybrid occurring in open-pollinated seed collected from a pure *E. risdonii* female in the pure species stand in the same area as described above and in Chapter 6. A crossing diagram of the families within the trial is shown in Table 5.1. Pure species and first and second generation hybrids of known pedigree were all represented in the trial. For the purpose of analysis, all progeny from pure species crosses were pooled into either *E. risdonii* (R) or *E. amygdalina* (A) cross types. The A polymix (Apoly) and R polymix (Rpoly) were comprised of approximately equal mixtures of pollen from five trees of *E. amygdalina* and *E. risdonii* respectively. The RApolymix was comprised of a mixture of each of these polymixes (Apoly and Rpoly) in approximately equal proportions. Progeny from interspecific crosses were pooled, along with individual F_1 hybrids from RApolymixes into an F_1 hybrid cross type. Remaining progeny from RA polymix crosses were pooled into the pure species cross types (A or R) depending on phenotype (progeny from these crosses could only be pure A, R or F_1 hybrids). Advanced generation hybrids (F_1op) were open-pollinated progeny from intermediate (H) hybrid phenotypes within the natural hybrid zone and therefore only their female pedigree was known. All F_1op families were pooled into one F_1op cross type. Although there was only one F_2 family, it was considered as a separate cross type for analysis purposes.

The trial was arranged in 16 randomised blocks, with 72 trees per block and families randomly assigned to single tree plots within each replicate. Where there were greater than 16 individuals per family, progeny were equally partitioned between blocks and randomly assigned to non-contiguous single-tree plots within blocks. Families with large numbers of individuals had extra progeny allocated to unfilled block positions. Where there were less than 16 individuals per family, progeny were randomly assigned among blocks. The trial, containing over 1100 trees was planted on an ex-forest site near Copping, in south east Tasmania. Spacing was 3m within rows and 4m between rows. Details of trial establishment are given in Table 5.2.

Species richness

The presence or absence of 25 insect taxa was determined on each individual tree within the controlled cross field trial from the eighth to the twelfth of March 1994. In addition, the presence or absence of another 6 taxa were included from scorings at a different time in the same season. These were the presence/absence of: hymenopterous gall 1

(14-19/2/94); damage caused by *Mnesampela privata* (scored 6/9/93); damage caused by the scarab beetle *Heteronyx* sp. (9/12/93); lepidopteran leaf tiers (tier 1, 2 scored 15/11/93) and; of double-ended lepidopteran leaf miners, scored 4/10/93. Details of each taxa are given in Table 5.3 and Figure 5.1. Further details of the additional 6 taxa are given in the individual species responses section. In total, the presence/absence of 31 insect taxa was determined. Identifications were confirmed as separate taxa with the help of Dick Bashford, Forestry Tasmania and using samples identified as separate taxa by (Whitham *et al.* 1994). Further identifications were undertaken following (CSIRO 1991). The hymenopterous gall 1, leaf tiers 1 and 2 and hymenopterous gall 5 were also grown through to the adult form of their lifecycle, to aid in identification. Miner 1 probably contained a number of individual species but attempts to separate it into further taxa by growing larvae out proved unsuccessful.

Species richness was calculated by summing the presence (1) or absence (0) of all taxa in the field trial. For the individual taxa hymenopterous gall 1, tier 1, tier2 and miner 4, counts of the number of taxa were converted to presence (1) or absence (0) data before being added to the species richness total. Family residuals were not able to be normalised by any standard transformations (including log transformation). Pairwise comparisons were therefore obtained using the non-parametric proceedure NPAR1WAY. Arithmetic means were calculated for each cross type for graphical presentation.

Individual species responses

Individual species responses were also determined for all taxa by analysing presence/absence of individual taxa. Cross type means or the proportion of plants in each cross type on which an insect taxa was recorded, were obtained using the MEANS procedure in SAS (1992). Due to the non-normal nature of the residuals of individual tree observations, all pairwise comparisons were undertaken using the NPAR1WAY procedure of (SAS 1992). In some cases, there was insufficient information to obtain meaningful results and these taxa were excluded. Individual species responses, where a significant difference between cross types was found included leaf tier 1, leaf tier 2, weevil damage, double ended leaf miner, *Amorbus* damage, hymenopterous gall 1, hymenopterous gall 2, *Chrysophtharta decolorata, C. nobilitata,* hymenopterous gall 4, hymenopterous gall 5, (see Table 5.3, Figure 5.1). Hymenopterous gall 1, caused by a chalcid wasp, was relatively common in the field trial and its distribution was investigated further in Chapter 6.

The response of the F_1 hybrids to the insect taxa were subsequently classified as susceptible, resistant, dominant or additive with respect to the pure parent species following Fritz *et al.* (1994). The resistance of hybrids was classified as additive if the proportion of plants with insect taxa was intermediate to that of the parent species and not significantly different from the mid-parent value. If the hybrids had a significantly ($P<0.05$) greater proportion of plants with insect taxa than the host parent with the greatest number of insect taxa, then the hybrid was classified as susceptible. Hybrids were deemed resistant if there were a significantly lower proportion of hybrid plants with insect taxa than both parent species. If hybrids had a proportion of insect taxa at least as high as the most susceptible parent, and not signficantly different from that parent species, then they were classified as dominant (Fritz *et al.* 1994). In addition, based on the parent species, taxa were classified as generalists, if parents were not significantly different and, specialists if one or other parent species had a significantly greater proportion of plants with insect taxa (see Bernays and Graham 1988, Cates 1980, Fox and Morrow 1981, Freeland and Saladin 1989, Fritz *et al.* 1994).

Leaf toughness

Leaf toughness was measured on a subset of the field trial from 10-11/1/95. Families assessed were *Eucalyptus amygdalina* (A2 x Apoly), *E. risdonii* (R2 x Rpoly) and their F_1 hybrids (A2xR2, R2xA2) with 10 individuals per family sampled (see also the crossing diagram in Table 5.1). These families were chosen as they were well represented in the trial and allowed direct comparison of reciprocal crosses. A subset of the single second generation family (F_2) was also randomly chosen and measured for leaf toughness at the same time as the F_1 and pure species crosses. A week later (17/11/95), the total number of F_2 individuals within the trial had their young leaves measured, since only the young leaves showed significant differences at the cross type level. The total number of individual F_1 plants measured was 50, as this was the number surviving in the trial on the date of assessment. Both the early and late data assessments are presented.

Leaf toughness was measured in the field using a portable penetrometer (after Sands and Brancatini 1991), see Figure 5.2. Three leaf types were measured on each tree: i) a fully expanded leaf from the current season's foliage (old); ii) the youngest fully expanded leaf (medium); and iii) the youngest leaf, immediately behind the bud (young). Five replicates of each leaf toughness category were randomly measured across the canopy on each plant and these measurements were averaged. These averages were then used as the raw data for individual plants in the subsequent analysis. Individual tree residuals of average leaf toughness for each leaf type (young, medium and old), were not significantly different from normal (0.54, 0.11, 0.30 respectively; Shapiro-Wilk statistic, W, using the UNIVARIATE procedure in SAS (1992). Data were therefore analysed using the repeated measures analysis analysis in

the GLM procedure. The three leaf classes old, medium and young were included as dependent effects in the model, where cross type was the independent effect. Pairwise comparisons between cross types were undertaken using the contrast statement (F test).

The correlation between leaf toughness and insect abundance was investigated, particularly within the large single F_2 family (see trial details). Associations were also examined graphically for the presence/absence of all the insect taxa used in the determination of species richness in the hybrid trial and the leaf toughness measurements on the young leaves.

Leaf oils

Oil analysis was undertaken on the same subset of trees selected from the field trial as leaf toughness, including *E. amygdalina, E. risdonii,* their F_1 hybrid and the single F_2 family. Leaf samples for analysis were selected at random across the whole canopy. All chemical analyses were undertaken by Dr Haifeng Li.

Sample preparation, oil extraction and analysis followed Li (1993), Li *et al.* (1994). Only leaves from the current season were used for extraction. The leaf lamina was cut into 1-2cm^2 pieces and 100g of these pieces randomly selected for oil extraction. A separate but equivalent 100g sample was prepared and dried to constant weight at 50°C for sample moisture content determination. Fresh leaf samples were steam distilled, using a glass distillation unit, for 6h. The oils were collected and weighed and oil yield for each sample calculated on a dry weight basis ($g\ g^{-1}$). The oils were analysed by combined GC-MS as described by Li (1993), Li *et al.* (1994). Identification of individual compounds was based on published reference mass spectra (Sternhagen *et al.* 1974) and an 'in-house' library of reference spectra (see Davies 1990, Li *et al.* 1994). Essential oils were extracted and identified under the same conditions using a Flame Ionisation detector (FID) on Gas Chromatograph, to give relative peak areas for individual compounds (Li *et al.* 1994). Full details of the oil components quantified are given in Li *et al.* (1994).

Principal components analysis on all the oil composition data collected was obtained using the PCA procedure in (SAS 1992). The association between oil characteristics and insect damage, presence/absence, or insect numbers was investigated using the single large F_2 family (see methods for family details). Pearson's correlation coefficients were calculated and bivariate plots were produced independently between insect numbers and PC1, PC2 values from the oil analysis or each of the 31 separate oil components.

Results

Species richness

After 3 years of natural colonisation of the field trial, the pattern of insect richness was remarkably similar to that observed in the natural hybrid zone by Whitham *et al.* (1994) (Figure 5.3). The number of species colonising pure *E. amygdalina* genotypes was significantly greater than those colonising pure *E. risdonii* genotypes ($P<0.000$, Table 5.4). Species richness on the hybrids was significantly greater than that on either of the parental phenotypes ($P<0.04$). The F_1 hybrids had greater species richness than the advanced generation hybrids (either the F_1op or the F_2 hybrids, $P\leq0.02$).

Individual species responses

Individual insect taxa had varying responses to the different cross types within the hybrid trial (Figure 5.4). A greater proportion of the hybrid plants were colonised by leaf tier 1 and 3, miner 4 or had weevil damage than either pure parent species. Insect taxa that had intermediate or dominance responses to the hybrid cross types included the hymenopterous gall 1 and *Chrysophtharta decolorata*. The specific damage caused by these insect taxa (except *C. decolorata*) is shown in Figure 5.1.

The majority of the responses of the insect taxa to the hybrids were found to follow the dominance hypothesis (i.e. not significantly different from the most damaged parent, after Fritz *et al.* 1994). Of the 31 taxa surveyed, only 12 had significant differences in their distribution between cross types and these are described in Table 5.5. Four of the 12 were clearly identified as generalists, that is, the proportion of plants colonised by insects were not significantly different on *E. risdonii* when compared with *E. amygdalina*. All 4 generalists displayed a clear preference for the hybrids. Furthermore, the generalists leaf tier 1 and leaf miner 4 showed a clear preference for the F_1 rather than F_2 or advanced generation hybrids (Table 5.5). Species specific insect taxa, where a significant difference in insect taxa distribution between *E. risdonii* and *E. amygdalina* was determined, included 8 out of the 12 taxa. Of these taxa, *E. amygdalina* was preferred as a host in 7 out of 8 cases. The majority of hybrids showed a dominance response when the insect taxa were species specific, with a proportion of hybrid plants colonised by insect taxa which was not significantly different from the parent which had the greatest colonisation by insect taxa (Table 5.5).

Leaf toughness

Leaf toughness for old, medium and young leaves on the different cross types within the hybrid trial are shown in Figure 5.5. No significant difference was found in leaf toughness between all cross types for old and medium leaf types. Young leaves were, however found to be significantly different amongst cross types ($P=0.028$). In this leaf class, no significant difference between the two F_1 families was found ($P<0.145$), so

they were pooled to form a single F_1 cross type. *E. amygdalina* had significantly tougher young leaves than *E. risdonii* ($P < 0.027$, Table 5.6). The F_1 and F_2 hybrids were intermediate in their leaf toughness, when compared with the pure parent species.

The complete F_2 hybrid population, measured a week after the initial measurements, showed no distinguishable segregation in leaf toughness (see Figure 5.6). It appears likely that leaf toughness may therefore be inherited additively. No correlation (Pearson's correlation coefficient), between leaf toughness and the presence/absence of any insect taxa surveyed was determined (an example is given in Figure 5.7). There was clearly no relationship within the F_2 between young leaf toughness and distribution of the insect taxa scored in this experiment, even though significant differences between cross types were determined.

Leaf oils

There was a clear difference in the oil compostion between *E. amygdalina* and *E. risdonii* (Figure 5.8). The F_1 hybrids (both the *E. amygdalina x E. risdonii* and the reciprocal cross) were intermediate between the two pure species. *E. amygdalina* was the most distinct group, with the F_1 hybrids tending slightly more towards *E. risdonii*. The F_2 hybrids ranged almost completely between *E. amygdalina* and *E. risdonii* and had a much greater range and variability on the PC2 axis.

Of the 31 insect taxa and the 31 different oil components as well as PC1 and PC2 for oil composition, only one instance of oils being associated with insect distribution was determined. The presence of eudesmols in the extracted leaf oil was found to be associated with the presence of hymenopterous gall 1 (see Figure 5.9). Galls were present when no alpha-eudesmol was present in the leaf oils and absent where alpha-eudesmols were present. Hymenopterous gall 1 was sampled several times from the field trial, and for these extra samples (see Table 6.1), and for beta-eudesmol and alpha-eudesmol, the numbers of galls were either very low or zero, where any eudesmol was present in the leaf oils. This trend was the same for the presence on galls in the pure species *E. amygdalina* and *E. risdonii.*

Discussion

Species richness was found to be greatest on *E. amygdalina* when compared with *E. risdonii* in the field trial. This was consistent with results obtained by (Whitham *et al.* 1994) in the natural hybrid zone, both within the hybrid zone and in adjacent pure species stands (see Figure 5.3 i and ii). Hybrids all had a greater species richness than both parent species in the trial. In the hybrid zone, the H and AH phenotypes had greater species richness than both parental phenotypes (A, R). The RH hybrid was,

however, intermediate between the parent phenotypes. Morphologically, the F_1 hybrids from the trial matched the H phenotype in Figure 5.3 (ii), whereas a wide array of phenotypes (AH, H, RH) are included in the advanced generation hybrid category (F_1op and F_2, see Figure 5.3). The F_1 hybrids in the trial and the H phenotype in the natural hybrid zone elicited a similar response from insect taxa. These results indicated that the hybrids were genetically more susceptible to a greater number of insect species than pure species phenotypes, consistent with the conclusions from the natural hybrid zone (Whitham *et al.* 1994). It was likely that the alternative hypothesis given by Whitham *et al.* (1994), where stress at the species boundary may cause of increased hybrid susceptibility was not the predominant cause for the increased species richness on the *E. amygdalina x E. risdonii* hybrids.

Advanced generation phenotypes in the hybrid zone (AH and RH) were either at least as suceptible as the intermediate H phenotype (AH), or less susceptible (RH) but if combined, their mean would appear below the mean of the H phenotype. It has been suggested that hybrid susceptibility may be due to genetic recombination disrupting co-adapted gene complexes controlling resistance (Whitham *et al.* 1994). If this was the case, advanced generation hybrids would be expected to be more susceptible to pests than first generation (F_1) hybrids. Advanced generation hybrids in this trial had lower species richness than F_1 hybrids. These results therefore provided evidence against increased susceptibility being attributable directly to genetic recombination (Fritz *et al.* 1994, Whitham *et al.* 1994). It is possible, however, that the combination of parental genotypes in the F_1 causes a dilution of genetic mechanisms, where genes encoding resistance are still fully functional but are diluted to such a degree to be below a resistance threshold. In advanced generation hybrids, recombination may allow resistance to be restored above a required threshold.

The response of hybrids between *E. amygdalina* and *E. risdonii* to individual insect taxa appears to be variable. For the 4 generalists identified, hybrids were more susceptible and had greater numbers of insect taxa than pure parent species. For specialists, hybrids generally followed the dominance hypothesis, where numbers of insect taxa were not signficantly greater than the parent with greatest proportion of trees with insect taxa. No cases of hybrid resistance, at a cross type level was found.

A significant difference in leaf toughness between cross types was determined for the families sampled in the hybrid trial. However, this was only for the very young leaves and leaf toughness was not found to be correlated with the presence/absence of any insect taxa investigated within the single F_2 family. Many herbivore species feed preferentially on newly flushed eucalypt foliage (e.g. *Paropsis atomaria* Ohmart *et al.* 1987) and the toughness of leaves increases dramatically with age (Edwards and Wanjura 1990, Larsson and Ohmart 1988, Lowman and Box 1983). It is likely,

therefore that since no correlations were determined between the insect taxa and the toughness of the very young leaves, that toughness does not play a large part in host selection for the taxa investigated. However, the frequency of some taxa was quite low and particularly with leaf chewers such as the chrysomelid beetles, some correlation may have been evident with greater levels of infestation.

The oil compostion of *E. amygdalina* was distinct from that of *E. risdonii.* The oil composition in the single F_2 family ranged almost completely between the two parent species and although there was no evidence of clear segregation, the range, particularly on the pc2 axis was considerably more than both parents species. The F_1 hybrids were intermediate between the two parent species, although there was a slight bias towards *E. risdonii.* A bias towards *E. risdonii* was also detected in RAPD analysis of F_1 progeny between the same species (Sale *et al.* submitted). It is possible that *E. risdonii* may be expressing some dominance over *E. amygdalina* in its oil characteristics.

The presence of eudesmols in the leaf oils was the only instance where either leaf oils or leaf toughness correlated with the presence or absence of any of the 31 insect taxa investigated. Within the F_2 family, if eudesmols were present, hymenopterous gall 1 was either very low in numbers or not present at all. Beta-eudesmols have previously been shown to have anti-mite capabilites (Morita and Yatagai 1994). However, further work is required to determined if the presence of eudesmols was the direct cause of the absence of the hymenopterous gall 1 in this instance.

In conclusion, these results indicated that increased species in the natural hybrid zone appears to be at least partly due to increased genetic susceptibility of the F_1 and advanced generation hybrids to pests. The consistent increase in species richness on naturally occurring eucalypt hybrids highlights the potential role that hybrids and hybrid zones may play in the conservation of biodiversity in temperate forest ecosystems. The responses of individual insect taxa to the hybrids varied, and the most common responses to the hybrids, were: dominance, where hybrids were not signficantly more susceptible than the most susceptible parent species; and susceptibility, where hybrids were significantly more susceptible than both parent species. No case of hybrid resistance was found.

Table 5.1. The number of individuals per cross for the families incorporated in the *E. amygdalina x E. risdonii* hybrid trial near Copping, south east Tasmania. Parents and cross types included were: A, *E. amygdalina*; Apoly *E. amygdalina* polycross (pollen is pooled from many male parents); R, *E. risdonii*; Rpoly, *E. risdonii* polycross; O.P., open pollinated progeny; Advanced, open pollinated progeny from intermediate hybrid (H) phenotypes in the natural hybrid zone; F_2, selfed progeny from a putative *E. amygdalina x E. risdonii* first generation (F_1) hybrid, of unrelated pedigree. From RApoly crosses, either pure species or hybrid progeny were produced, and the number of hybrid progeny (H) is given in brackets next to the total number of progeny.

		males							
		A1	A2	Apoly	R1	R2	Rpoly	RApoly	O.P.
	A1				5		16		15
f	A2			15		10	18	49 (4H)	16
e	A3			27			1	42 (7H)	9
m	A4			33			18	60 (0H)	16
a	A5			1			1		5
l	A8								
e	A11								2
s	R1	1		2			3	0 (1H)	9
	R2		8	17			35		31
	R3						32	73 (17H)	12
	R4							8 (0H)	
	R5								3

Advanced 1	64	Advanced 4	48
Advanced 2	70	Advanced 5	70
Advanced 3	69	F_2	79

Table 5.2. Establishment details for the *Eucalyptus amygdalina* x *E. risdonii* hybrid trial, near Copping in SE Tasmania.

established	27/6/1991
latitude	42° 46'
longitude	147° 43'
soil	sand/clay duplex
geology	sandstone
rainfall	792mm
aspect	NW
slope	5°

Table 5.3. Description of the insect taxa scored for their presence or absence in the *E. amygdalina* x *E. risdonii* experimental field trial.

taxa	label	identification	plant organ affected
hymenopterous gall	hymenopterous gall 1	Hymenoptera	leaf
flat hymenopterous gall	hymenopterous gall 2	Hymenoptera	leaf
large psyllid gall	psyllid gall 1	Psyllidae	leaf
sperical hymenopterous gall	hymenopterous gall 3	Hymenoptera	leaf
miner 1	miner 1	Lepidoptera	leaf
miner 2	miner 2	unidentified	leaf
miner 3	miner 3	Lepidoptera	leaf
double-ended mine	miner 4	Lepidoptera	leaf
autumn gum moth damage	*M. privata*	*Mnesampela privata*	leaf
basket moth	basket moth	*Hypertropha* sp.	leaf
tier 1	tier 1	*Strepsicrates ejectana*	leaf
tier 2	tier 2	*Chlorodes boisduvalaria*	leaf
tier 3	tier 3	*Acrocercops* sp.	leaf
adult tier 1 moth	adult tier 1	*Strepsicrates ejectana*	none
weevil damage	weevil	*Gonipterus scutellatus*	leaf
gum tree scale	*Eriococcus* spp.	*Eriococcus* spp.	leaf and stem
terminal gall	hymenopterous gall 4	Hymenoptera	stem
mite gall	mite gall	Psylloidea	leaf
stem galls	hymenopterous gall 5	Hymenoptera	stem
bud galls	hymenopterous gall 6	Hymenoptera	bud
chrysomelid 1	*C. decolorata*	*Chrysophtharta decolorata*	leaf
chrysomelid 2	*C. aurea*	*Chrysophtharta aurea*	leaf
chrysomelid 3	*C. nobilitata*	*Chrysophtharta nobilitata*	leaf
chrysomelid 4	*C.*sp. 14	*Chrysophtharta* sp 14	leaf
chrysomelid 5	*P. porosa*	*Paropsis porosa*	leaf
chrysomelid 6	*P. tasmanica*	*Paropsis tasmanica*	leaf
scarab beetle	*Heteronyx* sp.	*Heteronyx* sp.	young buds
Amorbus damage	*Amorbus*	*Amorbus obscuricornis*	shoots
leaf hopper	leaf hopper	*Eurymela fenestrata* (Eurymelidae)	shoots
helena gum moth	gum moth	*Opodiphithera helena*	leaf
mantis	mantis	Mantidae	predator

Table 5.4. Significant differences between cross types for mean species richness. Overall, the cross type effect was highly significant (P<0.001). Probabilities were obtained from individual pairwise comparisons using the NPAR1WAY procedure of SAS (SAS 1992) (see Figure 5.3 ii, methods). Cross type codes are given in Figure 5.4.

	A	**F_1**	**F_1op**	**F_2**
A				
F_1	0.000			
F_1op	0.033	0.000		
F_2	0.009	0.017	0.283	
R	0.000	0.000	0.000	0.000

Table 5.5. Summary of individual species responses observed in the controlled-cross hybrid trial between *Eucalyptus amygdalina* and *E. risdonii.* Taxa were either classified either as species specific (S) followed by species preferred or as generalists (G, see methods). Further, the F_1 hybrids response was classified as A or S (additive or susceptible, see methods), with the significance of the hybrid susceptibility following the classification. In the last column, comparisons of the susceptibility of first generation hybrids (F_1) versus advanced generation hybrids (F_1op) are made; the most susceptible cross is given, followed by the significance level (* = 0.05, ** = 0.01 and *** = 0.001). Details of taxa identification are given in Table 5.3. Taxa that had no significant difference between cross types were not included in this table.

species description	species specific or generalist	hybrid response (A, S)	F_1 vs advanced generation
leaf tier 1	G	S(***)	F_1**
weevil	G	S(***)	n.s.
miner 4	G	S(***)	F_1*
leaf tier 3	G	S(**)	n.s.
Amorbus sp.	S-*E. risdonii*	D	n.s.
hymenopterous gall 1	S-*E. amygdalina*	D	n.s.
C. decolorata	S-*E. amygdalina*	D	n.s.
C. nobilitata	S-*E. amygdalina*	D	n.s.
hymenopterous gall 2	S-*E. amygdalina*	A	n.s.
hymenopterous gall 4	S-*E. amygdalina*	A(n.s.)	n.s.
hymenopterous gall 5	S-*E. amygdalina*	A(n.s.)	n.s.
Chrysophtharta. sp 14	S-*E. amygdalina*	D(n.s.)	n.s.

None resistant

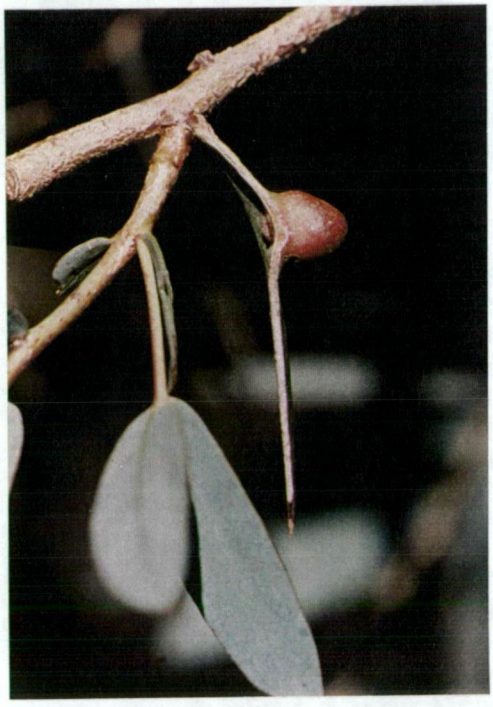
Table 5.6. Significant differences between cross types for mean leaf toughness on young leaves. Overall, the cross type effect was significant ($P<0.028$). See methods for how probabilities were obtained.

	A	F_1	F_2
F_1	0.026		
F_2	0.003	0.234	
R	0.027	0.665	0.618

Figure 5.1. Examples of individual species responses to the different cross types in the *E. amygdalina x E. risdonii* hybrid trial, shown below.

i) hymenopterous gall 1

ii) flat hymenopterous gall



iii) large psyllid gall (Psyllidae)

iv) spherical hymenopterous gall

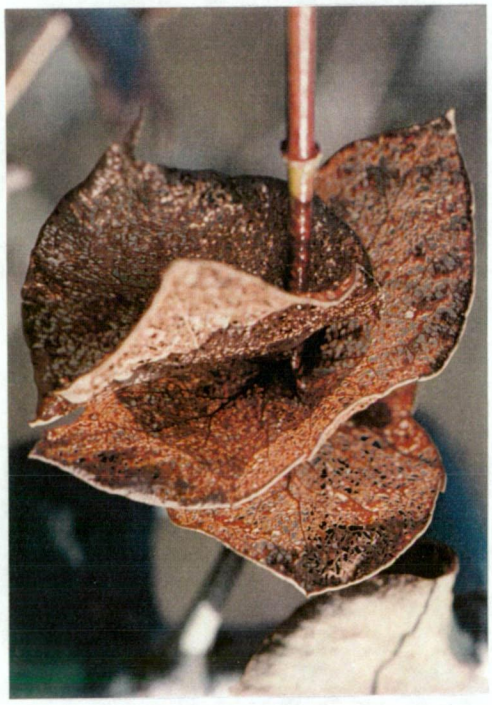
v) miner 1 (Lepidoptera)

vi) miner 2

vii) miner 3 (Lepidoptera)

viii) miner 4 (Lepidoptera)

ix) damage caused by autumn gum moth (*Mnesampela privata*) on *E. risdonii.*



x) close up of *M. privata* damage showing characteristic skeletonising

xi) basket moth (*Hypertropha* sp.)

xii) tier 1 damage

xiii) tier 1 adult

xiv) tier 2 damage

xv) tier 3 damage

xvi) weevil damage (*Gonipterus scutellatus*) showing characteristic tracks in leaf, early instar larvae (bottom of plate) and egg capsule (top)

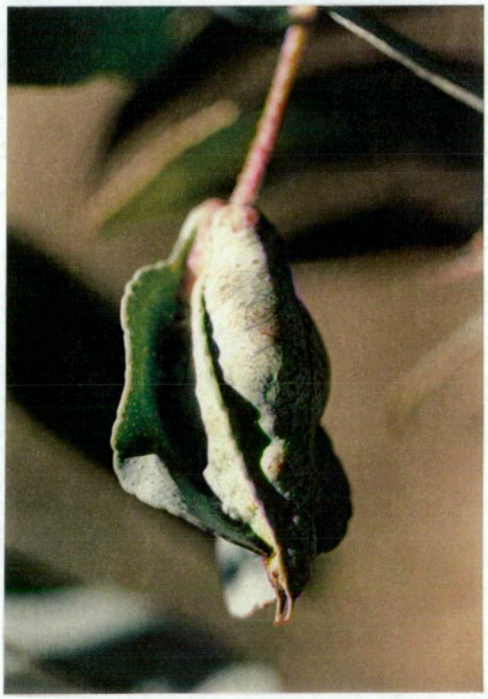
xvii) gum tree scale on stems (*Eriococcus* sp. 1)

xviii) gum tree scale on leaf lamina (*Eriococcus* sp. 2)

xix) hymenopterous gall 4 (Hymenoptera)

xx) mite gall

xxi) hymenopterous gall 5 (Hymenoptera)

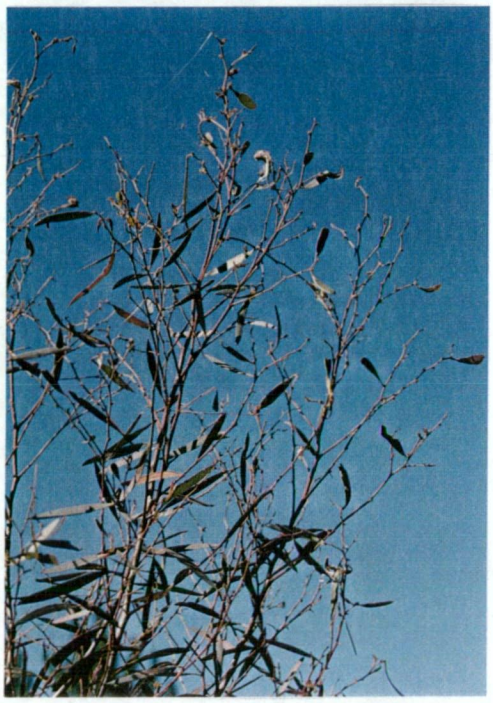


xxii) hymenopterous gall 6 (Hymenoptera)

xxiii) chrysomelid 5 (*Paropsis porosa*)

xxiv) chrysomelid 6 (*Paropsis tasmanica*)

xxv) scarab beetle (*Heteronyx* sp.)



xxvi) damage caused by *Heteronyx* sp.

xxvii) *Amorbus obscuricornis* nymph showing characteristic tip damage.

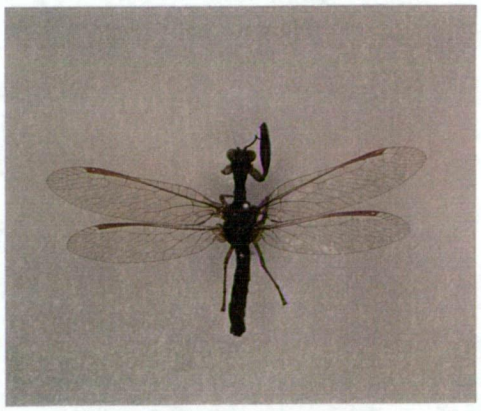
xxviii) *Amorbus obscuricornis* adult showing characteristic tip damage.

xxix) leaf hopper (*Eurymela fenestrata*)

xxx) helena gum moth

(*Opodiphithera helena*)

xxxi) mantis (Mantidae)



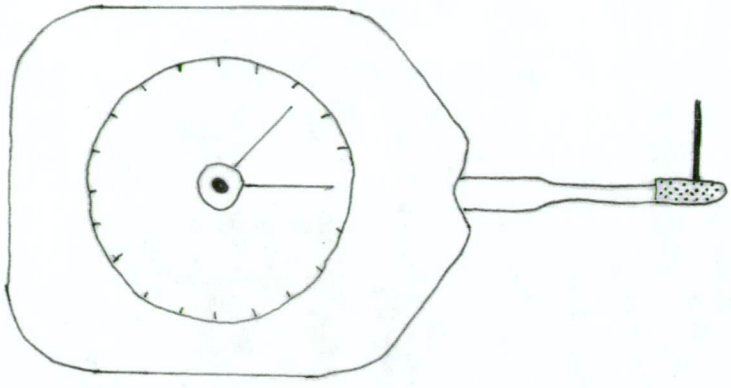


Figure 5.2. The portable leaf penetrometer used to determined leaf toughness in the field (adapted from Sands and Brancatini 1991).

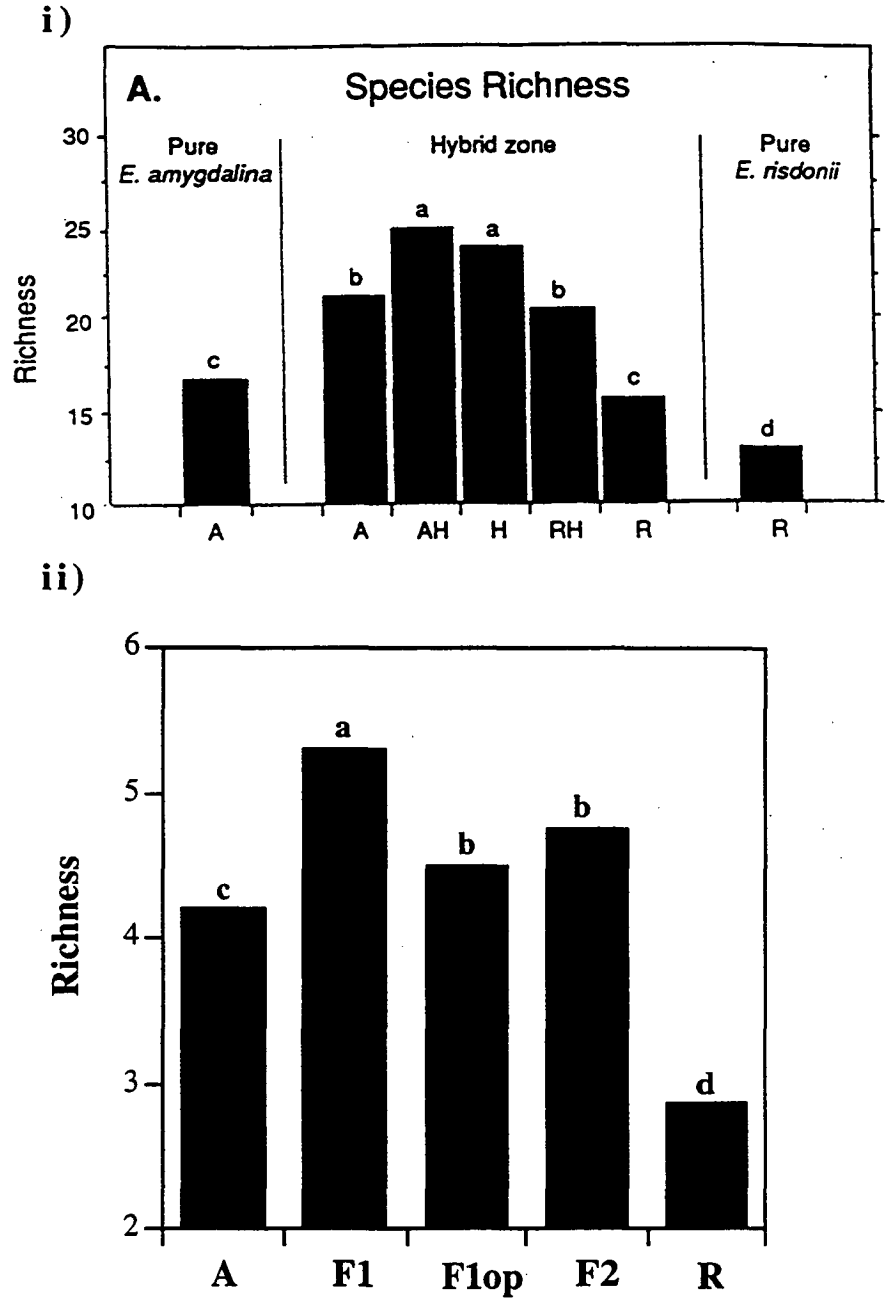


Figure 5.3. Average species richness (the number of insect taxa per individual tree) for i) the natural hybrid zone as determined by Whitham *et al.*(1994) for 40 insect and fungal taxa and; ii) within the *Eucalyptus amygdalina x E. risdonii* controlled-cross trial for 31 insect taxa (see methods). Significant differences at least at the 0.05 level are indicated by different letters (see Table 5.4, methods, Whitham *et al* 1994).

Note: cross-types are: A, *E. amygdalina*; R, *E. risdonii,* F_1, first generation hybrids; F_2, second generation hybrids (F_1 hybrid selfed); F_1op, advanced generation hybrids (see methods); H, intermediate hybrid phenoypte; AH, hybrid phenotype tending towards A; RH, hybrid, hybrid phenotype tending towards R.

Figure 5.4. Examples of individual species responses to the different cross types in the *E. amygdalina x E. risdonii* hybrid trial. i) leaf tier 2, ii) weevil, iii) *Amorbus*, iv) hymenopterous gall 1, v) *Chrysophtharta decolorata.* (See also Table 5.4). Different letters above the cross types represent significant differences at the 0.05 level (see methods)

Note: cross-types are: A, *E. amygdalina*; R, *E. risdonii*, F_1, first generation hybrids; F_2, second generation hybrids (F_1 hybrid selfed); Adv, advanced generation hybrids (see methods).

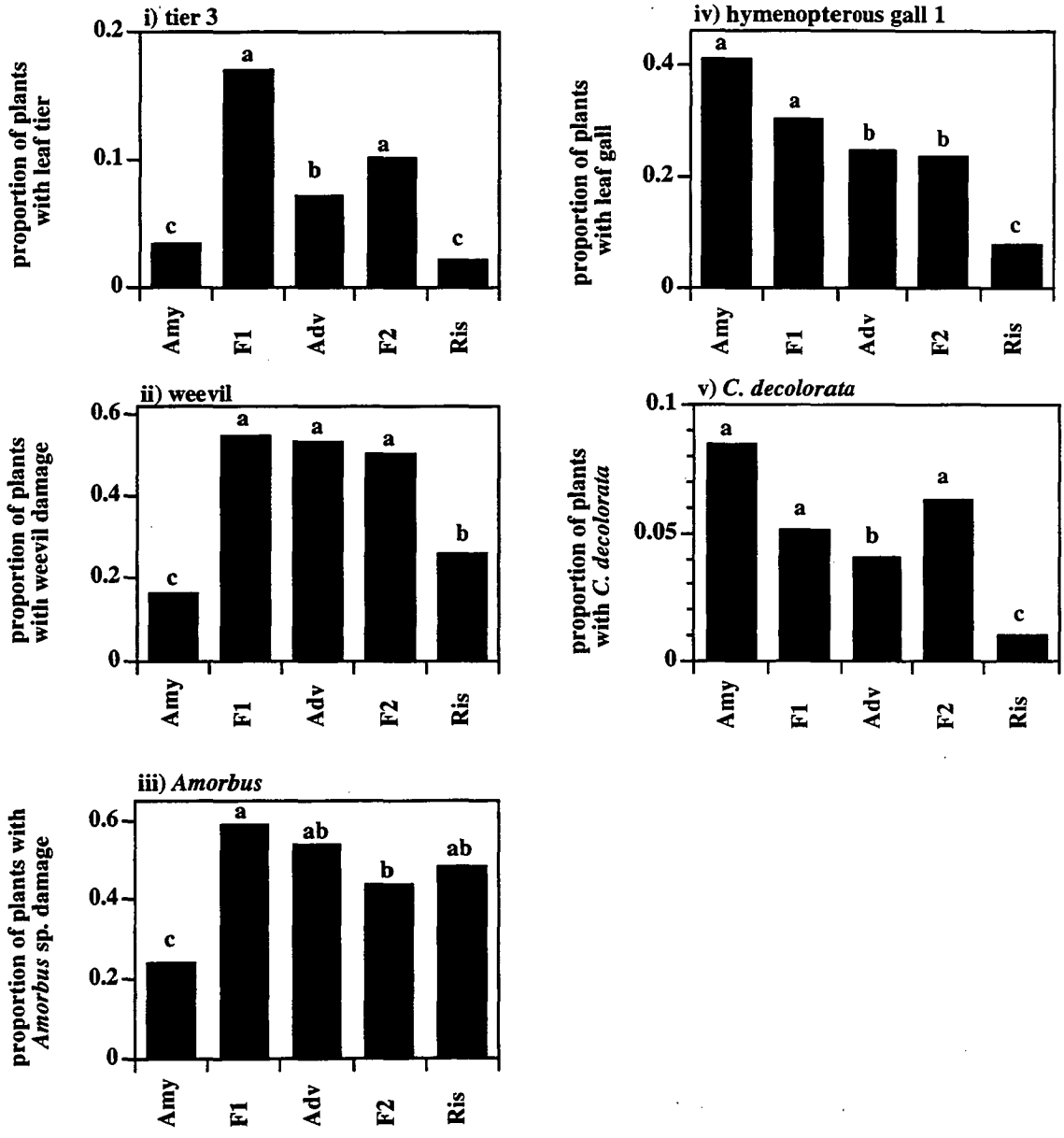
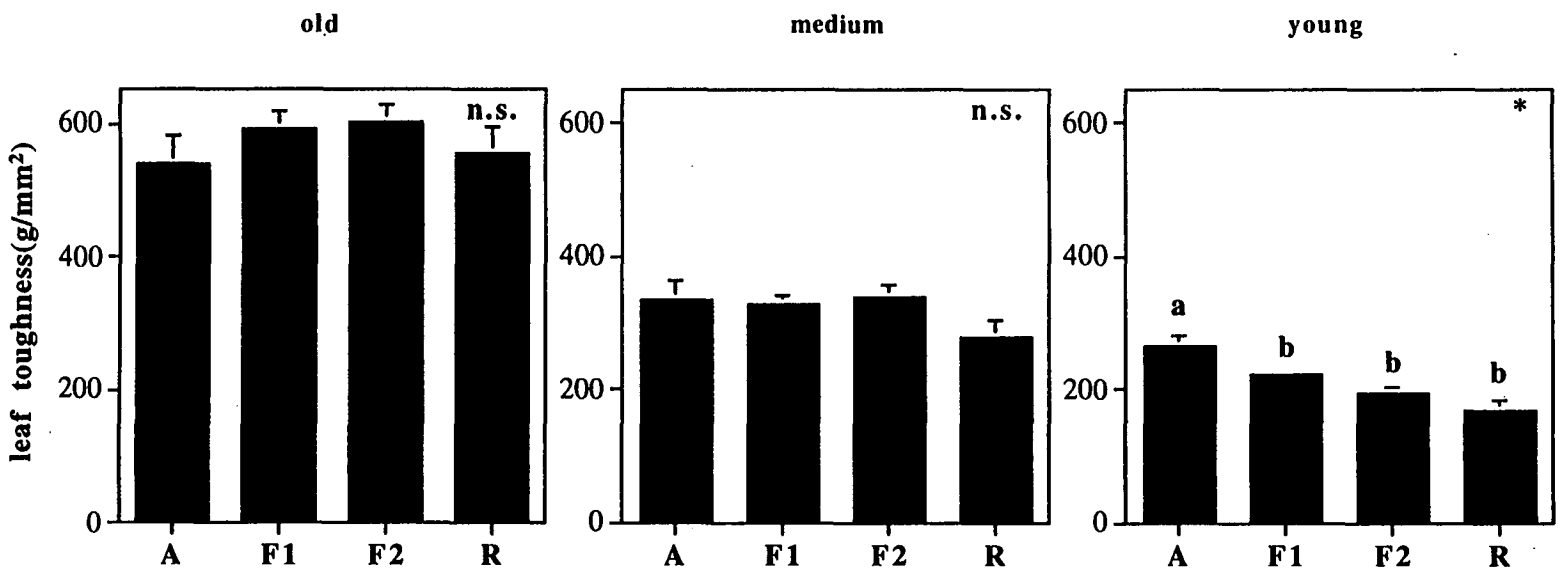


Figure 5.5. Leaf toughness (gmm^{-2}) of cross types within the experimental field trial, for leaf classes old (fully expanded leaf from current season's foliage), medium (youngest fully expanded leaf) and young (leaf immediately behind the bud). Overall significance of the cross type difference is given in the right hand top corner (F statistic from the GLM procedure in SAS (SAS 1992). Different letters above the columns represent significant differences at the 0.05 level.



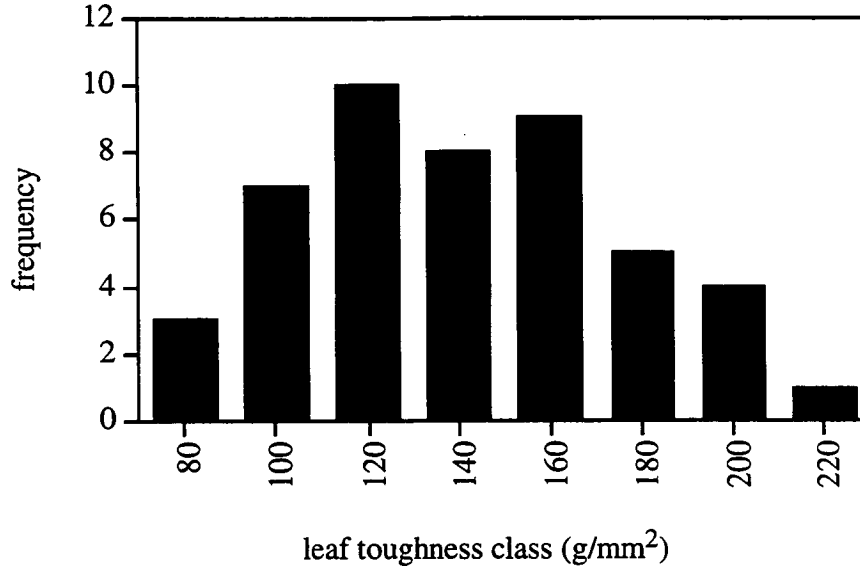


Figure 5.6. Frequency distribution of the leaf toughness of the young leaves of all the F_2 progeny within the experimental field trial. Classes shown are the upper limit, e.g. 100 = 80-100, 120 = 100-120 etc.

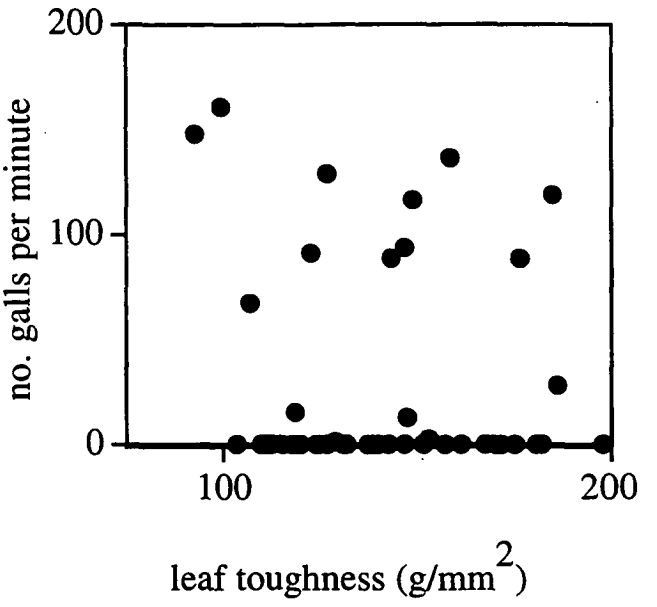


Figure 5.7. The relationship between leaf toughness and the number of hymenopterous galls on the 50 individual F_2 trees sampled in the hybrid trial (see methods).

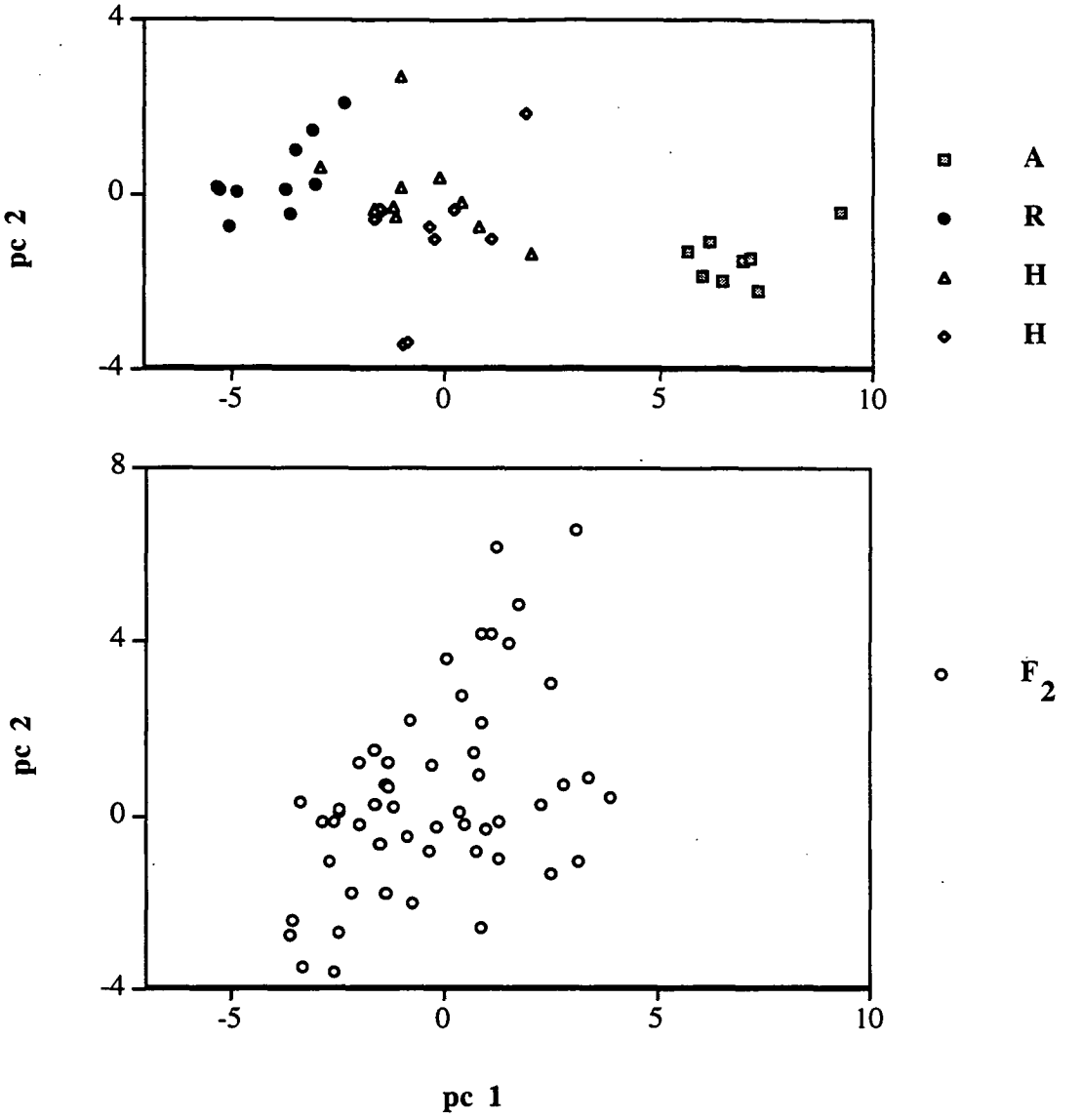


Figure 5.8. Principal components analysis of the oil component content of new seasons foliage of *E. amygdalina* (▣), *E. risdonii* (●), their F_1 hybrid *E. amygdalina x E. risdonii* (▲), the reciprocal cross *E. risdonii x E. amygdalina* (◆) and, on the separate graph, the F_2 hybrids.

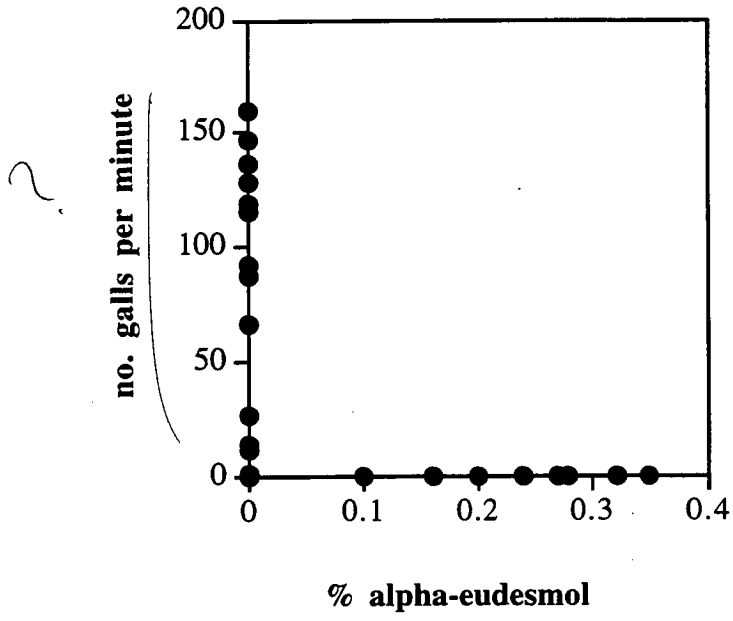


Figure 5.9. Distribution of hymenopterous gall 1 with respect to the presence of alpha-eudesmol for the 50 individual F_2 hybrid trees sampled in the hybrid trial (see methods).

Chapter 6

Hybrid susceptibility: host preference of a gall forming wasp on *Eucalyptus amygdalina x E. risdonii* hybrids

Introduction

In their study of the natural hybrid zone between *E. amygdalina* and *E. risdonii*, (Whitham *et al.* 1991a, Whitham *et al.* 1994) found that five of the 40 pest species examined were almost entirely restricted to the hybrid zone. In particular, one species of chalcid wasp was found to be 260 times more abundant on the average hybrid than on the parental phenotypes in adjacent pure species stands (Whitham *et al.* 1994). Of the 60 trees examined in both pure species zones, just seven galls produced by this wasp were observed and these were on the same leaf. In contrast, 1398 galls were recorded from 31 of the 46 hybrid trees examined. This species was given as an example of a taxon which was effectively restricted to the hybrid zone, and they suggested that numbers of this galling species could be influenced by loss of the hybrid zone.

The present study investigates the repeatability of this result for the same chalcid wasp, referred to as hymenopterous gall 1 in Chapter 5. The same trees assessed by Whitham were re-assessed, and other sites in the vicinity of the natural hybrid zone were surveyed. In addition, the distribution of galls was examined in the experimental field trial described in Chapter 5.

Materials and Methods

Natural hybrid zone

The natural hybrid zone between *Eucalyptus amygdalina* and *E. risdonii* has been well documented (see Potts 1986, Potts and Reid 1985, Potts and Reid 1988). Detailed climatic and ecological descriptions of this area are given by Kirkpatrick and Nunez (1980) and Nunez (1980). Permanent transects in and around this natural hybrid zone were established as a result of earlier work by Whitham *et al.* (1991b) and Whitham *et al.* (1994). Transects ran within the hybrid zone and in both the adjacent pure species stands of *E. amygdalina* and *E. risdonii.* Transect locations are given in Figure 6.1. Within the hybrid zone, Whitham *et al.* (1994) subjectively classified trees into five phenotypic classes. Trees were designated (A) if they resembled pure *E. amygdalina* and (R) if they resembled pure *E. risdonii.* Hybrids were classified as intermediate, resembling the F_1 phenotype (H), deviating towards

E. risdonii (RH), or deviating towards *E. amygdalina* (AH). This classification was verified using measurements of the juvenile and adult leaves, seed capsules and glaucousness (see Whitham *et al.* 1994). The hybrid zone transect included 75 trees in 15 blocked sets of *E. risdonii* (R), *E. amygdalina* (A), intermediate hybrid phenotypes (H) and hybrids resembling the backcross phenotype to *E. risdonii* (RH) and *E. amygdalina* (AH). In addition to the transect in the hybrid zone, permanent transects in the pure *E. risdonii* and *E. amygdalina* populations were set up by (Whitham *et al.* 1994), see Figure 6.1). Both pure species transects incorporated 20 trees of either *E. risdonii* or *E. amygdalina.* The same transects were used in this study. All permanantly marked transects are subsequently known as transects at Site 1 (see also Figure 6.1).

In addition to monitoring gall populations in the permanant transects, a transect near the hybrid zone at Site 1 but further down the hill, was investigated. It was located at the *E. risdonii* and *E. amygdalina* species boundary in an area where hybrids were very rare and corresponded to the ecotone site described by Potts (1986). This second site was chosen specifically to determine whether the presence of galls at the plant species boundary was dependent on the presence of hybrids. Pairwise samples of twenty trees of both *E. risdonii* and *E. amygdalina* were taken at this site. This site is subsequently referred to as the ecotone at Site 1 (see Figure 6.1).

A number of new unmarked transects were also surveyed at a second site (Site 2). These transects were undertaken to investigate the repeatability of results between hybrid zones in the same area. Site 2 was located at a seperate, smaller hybrid zone between *E. amygdalina* and *E. risdonii* further down the hill (SE) and isolated from the hybrid zone at Site 1 (see Figure 6.1). The transects undertaken in and around the smaller hybrid zone included i) pure *E. risdonii,* ii) pure *E. amygdalina* and, iii) in the hybrid zone itself. In the hybrid zone, a tree of each phenotypic class (A, AH, H, RH, R) was sampled in groups at 15 positions along the ridge line. In the pure *E. amygdalina* population, and in the pure *E. risdonii* population, 20 trees were sampled in each transect. Identification of the 5 different phenotypes for these additional transects was based on field morphology.

Two minute counts for the number of individual hymenopterous leaf galls (hymenopterous gall 1, see Table 6.3) were undertaken on the trees in the permanant transects at Site 1, (scored 8/3/94-15/3/94) and in the transects at Site 2 (scored 11.4.94). All gall counts were determined from an even scan over the entire canopy of each individual tree and later converted to counts per minute. This gall was chosen to be assessed for a number of reasons. Firstly, this gall had been assessed previously at Site 1 and was found to be virtually restricted to the hybrid zone

(Whitham *et al.* unpublished data). Secondly, the highest gall numbers were found on the hybrid phenotypes within the hybrid zone and; finally the gall was reasonably common in the natural hybrid zone, at both Site 1 and Site 2 and in the controlled cross trial (see below). The presence of the gall at all the sites gave an opportunity to investigate the genetic basis behind the pattern of gall distribution. In particular, the genetic nature of the apparent hybrid susceptibility was able to be examined.

Isolated hybrid phenotypes, thought to be the products of long distance pollen dispersal (Potts and Reid 1988), were found within the pure *E. amygdalina* (A) population at Site 2. When hybrids were located within the *E. amygdalina* population, the hybrid and the closest *E. amygdalina* were scored. In total, 22 paired samples were scored in this manner. These paired counts were undertaken primarily to determine if hybrid phenotypes in isolation were also susceptible to galls as was previously shown in the hybrid swarm (Site 1) by Whitham *et al.* (1994). Exactly the same trees were used in the assessment of Site 1 that were used by Whitham *et al.* (1994), except for the ecotone, which had not been previously sampled.

Phenotype residuals of individual hymenopterous gall 1 counts were unable to be normalised by most standard transformations including the log transformation used by Whitham *et al.* (1994). Data were therefore split into categories: zero galls (0), some galls (1-50) and lots (>50). These data were then analysed using the CATMOD procedure in SAS using cumulative logits (for ordinal data) with the response function and the phenotype effect in the model (SAS 1992). Individual pairwise comparisons between phenotypes were undertaken using the contrast statement in the CATMOD procedure.

Field trial

Counts of the number of individual hymenopterous leaf galls (hymenopterous gall 1, see Table 6.3) were undertaken on all the trees in the hybrid trial detailed in the previous Chapter. Determining the distribution of hymenopterous gall 1 in the hybrid trial allowed the direct comparison with the gall's distribution in the natural hybrid zone and therefore an investigation into the extent that any observed susceptibility was due to genetic susceptibility. Gall counts were undertaken four times, on 9/93 (sample 1), 11/93 (sample 2), 2/94 (sample 3) and 2/95 (sample 4). The aim of the different samples was to investigate the gall distribution both within and between seasons and determine the consistency of different assessment techniques. Counts were undertaken using a number of different methods. These included timed counts of 30 seconds, for samples 1, 2 and 3 (60 seconds for sample 4), of: i) the number of individual galls and, ii) the number of leaves infested with

the galls. In addition, the total number of galls on each tree was determined (iii). All gall counts were later converted to the number of galls or leaves with galls per minute. Details of the gall counts undertaken in each sample are given in Table 6.1.

Data that were normal (based on cross type residuals), were analysed using the GLM procedure in SAS (SAS 1992) using the following model:

trait = replicate + cross + family (cross) + error.

Where replicate is the replicate number, cross is the cross type and both were treated as fixed effects . Family was nested within cross type and treated as a random effect. Individual pairwise comparisons were undertaken using the contrast statement in GLM. Traits analysed using GLM included leaf count 1, leaf count 2, leaf count 3, and total galls 3.

Traits that were not normal included total galls 2, gall count 3 and gall count 4. These data were subsequently divided into categories: zero galls (0), some galls (1-50) and lots (>50) and then analysed using the CATMOD procedure in SAS using cumulative logits (for ordinal data) with the response function and cross type effect in the model. Pairwise comparisons between cross types were undertaken using the contrast statement.

Results

Natural hybrid zone

The distribution of the hymenopterous leaf gall 1 has been determined previously in 1990 by Whitham (unpublished data, see Figure 6.2 i). The distribution of the hymenopterous gall 1 at Site 1, both within the hybrid zone, in pure species stands and in the ecotone for this more recent survey is given in Figure 6.2 ii.

At site 1, the hybrid phenotypes H and RH had the greatest number of galls (Figure 6.2 ii). There was no significant difference in the number of galls on *E. amygdalina* (A) or *E. risdonii* (R), either in the ecotone or pure species stands, (Figure 6.2, Table 6.6 i). However, there was a tendency for *E. risdonii* to have slightly greater gall numbers than *E. amygdalina,* and *E. risdonii* from the hybrid zone had a significantly ($P<0.05$) greater number of galls than *E. amygdalina* in the pure species stand. This trend was also noted earlier in the 1990 assessment by (Whitham *et al.* 1994; see Figure 6.2 i). There was also a tendancy for gall numbers to be higher on pure species phenotypes within the hybrid zone when compared with pure species stands (A hybrid zone vs. A pure $P=0.014$; R hybrid zone vs. R pure, P=

0.056). There was no significant difference in gall counts on pure species phenotypes at the ecotone or hybrid zone site. Within the hybrid zone there was significantly ($P<0.05$) greater number of galls on the RH compared with the R phenotype, but no significant difference occurred between A and AH. Generally, results obtained from the permanant transects at Site 1 agreed with Whitham's earlier findings.

Gall abundance was markedly higher at Site 2 when compared with Site 1 (Figure 6.3). While galls were virtually absent from the pure *E. amygdalina* population at Site 1, the numbers of galls on *E. amygdalina* at Site 2 approached the maximum mean number of galls observed on any phenotypic class at Site 1. While there was only a slight trend toward *E. risdonii* as the preferred host at Site 1, at Site 2 the galls exhibited a clear preference for *E. risdonii* phenotypes, although this was only significant for the pure species sample (R pure vs A pure $P= 0.015$; R pure vs. A $P= 0.012$). While the H and RH phenotypic classes had the highest abundance of galls at Site 2, as was observed at Site 1, the levels were not significantly greater than gall numbers on the pure R phenotypes in the hybrid zone.

Although responses differed from Site 1 to Site 2, there were a number of observations that were consistent. Firstly, backcross hybrids always had more galls than the equivalent parent phenotype within the hybrid zone. Secondly, it was always a hybrid phenotype (whether it was H or RH) which had the greatest numbers of galls.

Paired samples undertaken from isolated hybrids near the pure *E. amygdalina* transect showed that the averaged hybrid phenotypes (H all, Figure 6.3 ii) had significantly more galls than the *E. amygdalina.* When the hybrids were partitioned into phenotypic classes, the H and RH hybrid classes also had a significantly ($P < 0.05$) larger number of galls than adjacent *E. amygdalina* (A) trees. Counts on the isolated hybrids were as high as those from comparable phenotypes from the hybrid zone and pure species samples of *E. risdonii* at Site 2.

Field trial

Significant differences ($P<0.001$) between cross types were observed for three of the seven assessments undertaken (Table 6.3). Within the field trial, the hymenopterous gall 1 was distributed preferentially on *E. amygdalina* when compared with *E. risdonii* (Figure 6.4, Table 6.4). This occurred independently of sample method and time of sampling (Figure 6.4, Table 6.4). However, this difference was significant only for total galls 2, gall count 3 and gall count 4. Sampling the gall population using leaf counts gave a significant difference between cross types in one out of

three samples (n.s., n.s. and *, Table 6.3). Timed gall counts and total gall counts generally appeared to distinguish the difference between cross types to a greater extent. It was notable that the first total gall count gave both higher gall numbers and a highly significant difference between cross types ($P<0.001$, Figure 6.4 iii), whereas the second total gall count showed no significant difference between cross types ($P=0.078$, Figure 6.4 vii), and gall numbers were lower (except for F_2). This difference is probably due to the loss of infected leaves, particularly on *E. amygdalina* over the 93/94 summer season (Figure 6.4 i versus 6.4 iv)..

The hybrid cross types F_1 and F_1op had gall numbers that were consistently intermediate between the two parent species A and R. The single F_2 family however, quite often had the greatest number of galls when compared with the other cross types and while it was not significantly more susceptible in all cases, it always had significantly more galls than *E. risdonii* (see Table 6.4).

Discussion

The distribution of hymenoterous gall 1 in the natural hybrid zone at Site 1 was remarkably similar to that determined by Whitham *et al.* (1994). At this site at least, it appeared that results were repeatable between years (1990 versus 1993/94). (Whitham 1989), similarly found that over 6 years, the trees in a *Populus* hybrid zone supported far more aphids than the pure host species. While gall numbers changed, the censuses consistently showed that the hybrid zone was the centre of gall abundance. Similarly, over two years in a natural hybrid zone between *Quercus gambelii* and *Q. grisea* in New Mexico (USA), the density of the leaf mining moth *Phyllonorycter* was consistently higher on *Q. gambelii* (Preszler and Boecklen 1994). Hybrid phenotypes also had consistently intermediate miner densities. The repeatability of host preference patterns between years at the same site, as was determined here, appears to be generally supported.

In the present study, marked differences were observed between the two hybrid zones sampled in the same year. At the second site, the numbers of galls on *E. risdonii* were much higher than *E. amygdalina* and infact were not significantly different from cross types with the highest gall numbers (H and RH). There are a number of possible hypotheses which may explain this difference. Firstly it is possible that the isolated population of *E. risdonii* at this second site may be genetically different from the *E. risdonii* at Site 1, since the leaf shape at Site 2 is not as broad (Potts, pers. comm.). The entire population of *E. risdonii* at Site 2 may therefore be introgressed at this site, although local adaptation or genetic drift can not be excluded. A genetic difference between the two *E. risdonii* populations could

cause a different response by the gall to the population at Site 2. Secondly, for the *E. risdonii* host preference to be expressed, gall numbers must be high, or at least higher than numbers found at Site 1. The gall forming wasps may need to be undergoing a local population explosion before *E. risdonii* is utilised as a host to its full extent. Thirdly, the distribution of the gall between sites may be extremely patchy which perhaps could have contributed to the fact that the numbers of galls were almost doubled at Site 2 when compared with Site 1. However, the fact that the hybrid phenotypes surveyed in isolation in the *E. amygdalina* population, were also found to have significantly higher numbers of galls indicates that the susceptibility of hybrids to the galls is not limited to hybrid zones and, furthermore could not be directly attributed to the presence of the hybrid zones themselves.

In the field trial, *E. amygdalina* was found to be the preferred host species of hymenoterous gall 1 when compared with *E. risdonii*. This was the reverse of the pattern determined in the natural hybrid zone at both Sites 1 and 2 and is difficult to explain. It is possible that the gall producing wasps in the trial are preconditioned to *E. amygdalina*. The forest surrounding the trial was dominated by *E. amygdalina*, although there was also a small population of *E. tenuiramis* (a species closely related to *E. risdonii*) in the area. Secondly, there may be marked genotype x site interactions associated with susceptibility. This may arise through a differential in plant stress levels, growth patterns or nutrient status. Physical factors such as water and light availability, temperature, and nutrient status of different sites are important in the distribution of eucalypts in Tasmania (Davidson and Reid 1985, Davidson and Reid 1987, Davidson and Reid 1989, Kirkpatrick and Marks 1985, Williams and Potts 1996). *E. risdonii* is usually found on mudstone and *E. amygdalina* on sandstone in south east Tasmania (Williams and Potts 1996). The field trial was established on a sand/clay duplex over sandstone. The drainage of the site, because of the presence of the clay was probably not as good as drainage on most sandstone soils. It is possible that this lack of drainage may have contributed to the *E. amygdalina* being more stressed at this site and therefore attracting greater gall numbers than *E. risdonii*. However, *E. risdonii* was also planted on a soil type on which it is not usually found although this eucalypt species may have a higher tolerance to stress (Potts and Reid 1985). Further factors such as different mycorrhizal associations may affect host fitness and therefore host choice by the wasp (Gehring and Whitham 1994). Tree phenology has been linked to the susceptibility of *Quercus* to galling wasps (Csoka 1994), and it is possible that phenological differences between sites may also have contributed to the different host selection.

Finally, the gall producing wasp may have been genetically different at the trial site compared with the natural hybrid zone, since the two sites were quite separate geographically. Genetically different wasps could react differently to host choice between *E. amygdalina* and *E. risdonii.* It is unlikely that the gall forming insect is a completely different species at the two sites. Galls induced by different insects are generally species specific and are often used in taxonomic identifications (Floate *et al.* 1996, Gagné 1989, Palmer 1952 and references therein). Given that the structure of hymenopterous gall 1 was consistent and highly characteristic, and that wasps which were raised from *E. amygdalina, E. risdonii* and hybrid leaves at both sites appeared to be identical, it is likely that this gall is initiated by one species only.

Hybrids in the controlled-cross trial generally had intermediate numbers of the hymenoperous gall 1 when compared with *E. amygdalina* and *E. risdonii.* Morrow *et al.* (1994), similarly found a similar intermediate response of the gall to the hybrids for an unidentified hymenopteran stem gall in a *E. obliqua x E baxteri* hybrid zone. This trend also agreed with the total gall load in a *Quercus* hybrid zone, supporting the additive hypothesis, with the hybrids supporting intermediate numbers of galls (Aguilar and Boecklen 1992, Boecklen and Larson 1994, Fritz *et al.* 1994). However, other trends were also noted for different gall species in Morrow *et al.* (1994) and for indivdual gall species by Boecklen and Larson (1994).

Floate and Whitham (1993), in their 'hybrid bridge' hypothesis, suggested that hybrids may morphologically, genetically and spatially bridge gaps between parental species and their presence may facilitate host shifting by herbivores in a series of gradual steps. They argued that herbivores could shift on to the new host species via these intermediates even though the plant species remain allopatric (Floate *et al.* 1993, Floate and Whitham 1993). Certainly, in the natural hybrid zone a morphological continuum exists between *E. amygdalina* and *E. risdonii* (Potts 1986, Potts and Reid 1985). However, both *E. amygdalina* and *E. risdonii* had a tendancy to have greater gall numbers in the ecotone when compared with pure species stands (although n.s. for *E. risdonii*). This suggested that the increased abundance of galls on the pure species phenotypes in the hybrid zone was not due entirely to the presence of hybrids alone. It also suggested that if any host shifting was occurring, it was unlikely to be due entirely to the presence of hybrids. Hybrids in general, including classes H, AH and RH, appeared to have the greatest gall numbers when they were found in isolation in a pure *E. amygdalina* population. Whitham (1989) suggested that hybrids may act as refugia when gall populations are low. In this study, it was possible that the hybrids were acting as habitat islands, where populations were low in the surrounding pure *E. amygdalina* populations.

Table 6.1. Details and descriptions of the different counts of the hymenopterous leaf gall 1 (See Table 5.3) in the *E. amygdalina x E. risdonii* hybrid trial.

trait	sample no.	trait name	date	description
leaf count 1	1	leaf galls 1	13/9/93	number of leaves with galls
leaf count 2	2	leaf galls 2	8/11/93	number of leaves with galls
total galls 2	2	total galls 2	8/11/93	total number of individual galls
leaf count 3	3	leaf galls 3	14/2/94	number of leaves with galls
gall count 3	3	gall count 3	14/2/94	number of individual galls/ minute
total galls 3	3	total galls 3	14/2/94	total number of individual galls
gall count 4	4	gall count 4	6/2/95	number of individual galls/minute

Table 6.2. Probabilities for pairwise comparisons between cross types for counts of the hymenoterous gall 1 in natural hybrid zones at **i)** Site 1, **ii)** at Site 2, and **iii)** for paired samples at Site 2. Probabilites were obtained from pairwise comparisons using the CATMOD proceedure in SAS (SAS 1992; see Figure 6.3, methods).

i)

	A(p)	**A(e)**	**A**	**AH**	**H**	**RH**	**R**	**R(e)**
A(p)								
A(e)	0.014							
A	0.014	0.922						
AH	0.003	0.282	0.356					
H	0.000	0.048	0.079	0.436				
RH	0.000	0.045	0.074	0.409	0.984			
R	0.016	0.989	0.915	0.290	0.050	0.047		
R(e)	0.064	0.330	0.316	0.053	0.004	0.004	0.354	
R(p)	0.310	0.048	0.310	0.006	0.000	0.000	0.056	0.236

ii)

	A(p)	**A**	**AH**	**H**	**RH**	**R**
A	0.980					
AH	0.421	0.434				
H	0.098	0.113	0.394			
RH	0.050	0.058	0.212	0.630		
R	0.073	0.087	0.323	0.904	0.706	
R(p)	0.015	0.012	0.081	0.374	0.744	0.437

iii)

	A	**AH**	**H**
AH	0.117		
H	0.000	0.155	
RH	0.012	0.355	0.765

H(all)	0.000

Table 6.3. Tests for the difference between cross types for each scoring of the hymenopterous gall 1 (see Table 6.4). F tests were obtained from GLM and the Chi-square statistic was obtained for ordinal data using the CATMOD procedure of SAS (1992). Details of tests are given in the methods.

trait	df	Chi-square^{x}/ F test†	Prob
leaf galls 1	4	1.88†	0.162
leaf galls 2	4	2.99†	0.049
total galls 2	4	56.79^{x}	0.000
leaf galls 3	4	1.38†	0.280
gall count 3	4	91.95^{x}	0.000
total galls 3	4	2.66†	0.078
gall count 4	4	46.40^{x}	0.000

Table 6.4. Pairwise comparisons between the five different cross types within the *E. amygdalina x E. risdonii* hybrid trial. Contrasts between cross types were derived using GLM (i, ii, iv, vi) or CATMOD (iii, v, vii). Note: A = *E. amygdalina*, F1 = *E. amygdalina x E. risdonii* first generation hybrids, F1op = open-pollinated progeny from intermediate hybrids, F2 = second generation hybrid.

i) leaf gall 1 n.s.

	A	F1	F1op	F2
F1	0.3365			
F1op	0.2114	0.9005		
F2	0.8378	0.2328	0.0918	
R	0.0662	0.5438	0.2736	0.0281

ii) leaf galls 2 (P<0.05)

	A	F1	F1op	F2
F1	0.9491			
F1op	0.7984	0.8944		
F2	0.0204	0.0377	0.0006	
R	0.5992	0.6967	0.6712	0.0025

iii) total galls 2 (P<0.001)

	A	F1	F1op	F2
F1	0.006			
F1op	0.000	0.943		
F2	0.008	0.108	0.085	
R	0.000	0.000	0.022	0.000

iv) leaf galls 3 n.s.

	A	F1	F1op	F2
F1	0.7142			
F1op	0.4809	0.8867		
F2	0.7041	0.4723	0.1901	
R	0.0819	0.2532	0.1187	0.0228

v) total galls 3 (n.s.)

	A	F1	F1op	F2
F1	0.9341			
F1op	0.7658	0.8827		
F2	0.1633	0.1912	0.0260	
R	0.3291	0.4420	0.3269	0.0114

vi) gall count 3 (P<0.001)

	A	F1	F1op	F2
F1	0.009			
F1op	0.000	0.864		
F2	0.002	0.357	0.243	
R	0.000	0.000	0.000	0.005

vii) gall count 4 (P<0.001)

	A	F1	F1op	F2
F1	0.006			
F1op	0.000	0.336		
F2	0.000	0.137	0.356	
R	0.000	0.000	0.000	0.000

Figure 6.1. The approximate location and layout of the two natural hybrid zones and pure *E. amygdalina* and *E. risdonii* stands. The permanant transects, marked transect 1A, 1R or 1H, correspond with *E. amygdalina* pure species transect, *E. risdonii* pure species transect, and the hybrid zone transect (Site 1). The approximate position of the unmarked transects are given by 2A, 2R and 2H for the *E. amygdalina*, *E risdonii* and the hybrid zone transects respectively (Site 2). The position of the ecotone (E), adjacent to the permanant hybrid zone transect at Site 1 is also shown (hereafter referred to as part of Site 1).

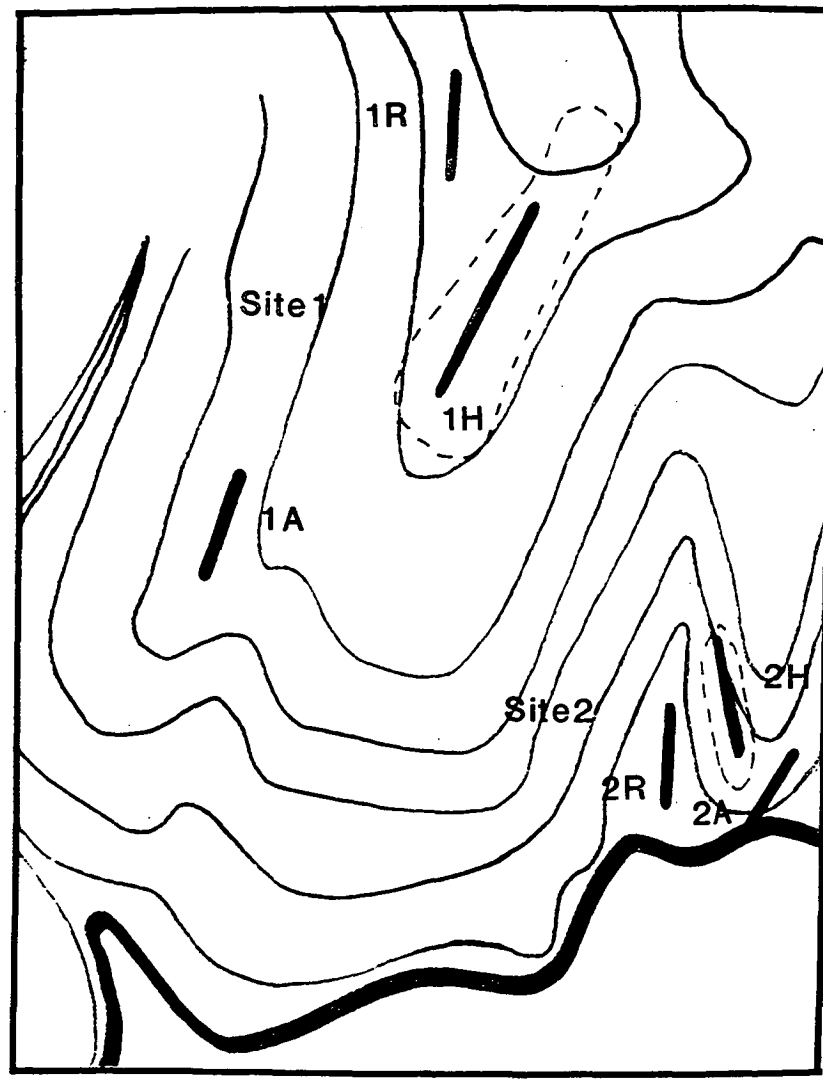
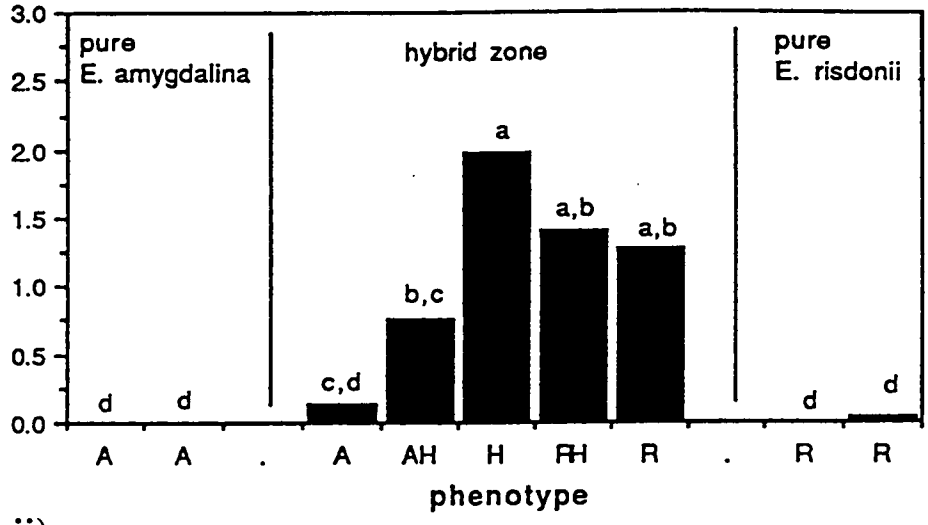


Figure 6.2. Mean number of hymenopterous gall 1 (per minute) counted on phenotypes at Site 1 within the natural hybrid zone and in adjacent pure species stands i) as determined in 1990 by Whitham *et al.* (unpublished data) and ii) as determined in March 1994 in the present study. Phenotypic categories represented are: A(p), *E. amygdalina* from an adjacent pure species stand; A(e), *E. amygdalina* from the ecotone; and from the hybrid zone A, *E. amygdalina* ; AH, resembling the backcross phenotype to *E. amygdalina*; H, intermediate or putative F1 phenotype in the hybrid zone; RH, advanced generation backcross phenotype; R, *E. risdonii* ; R(e), *E. risdonii* from the ecotone; R(p), *E. risdonii* from an adjacent pure species stand. Significant differences at the 0.05 level are indicated by different letters (see Table 6.2, methods, Whitham et al 1994).

i)



ii)

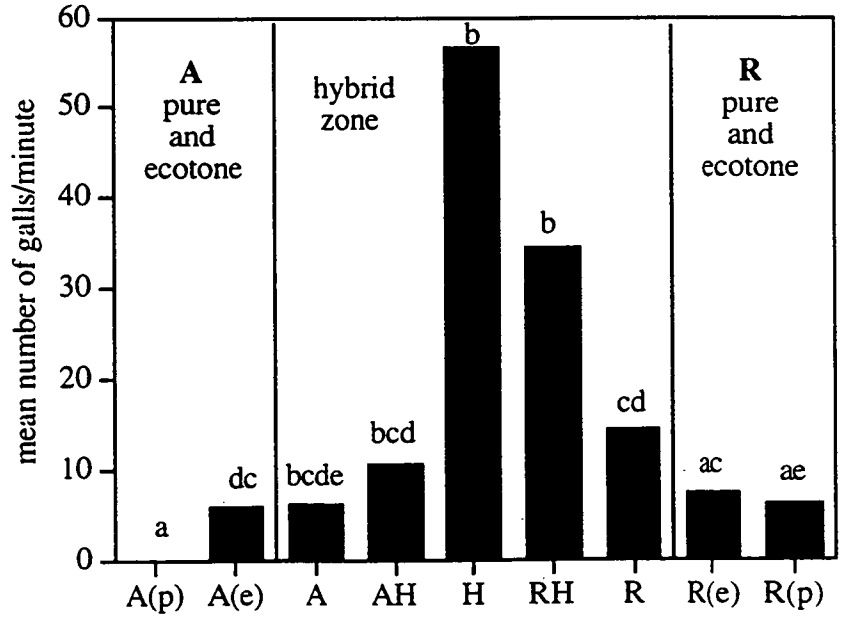
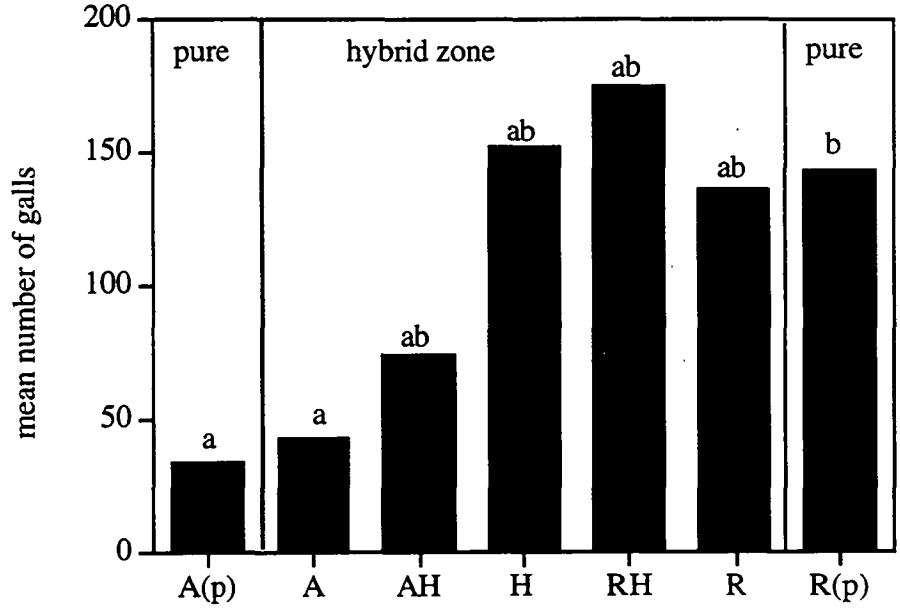
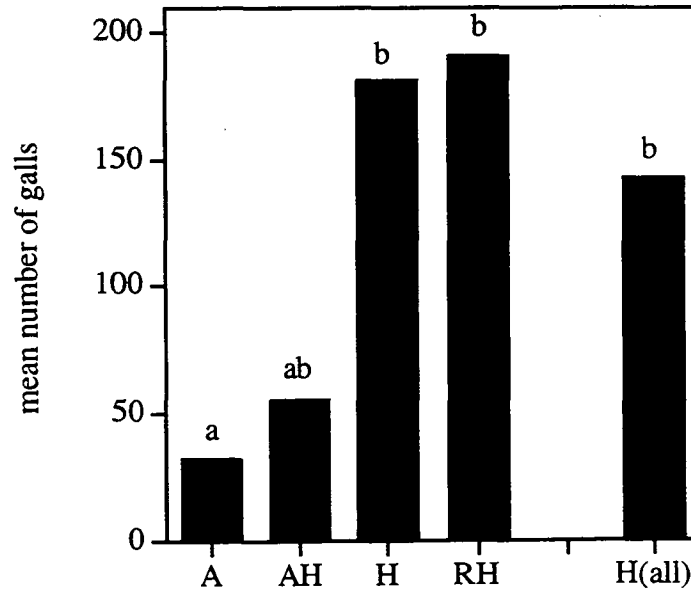


Figure 6.3. Mean number of hymenopterous gall 1 counted per minute at Site 2 **i)** in the hybrid zone and pure *E. amygdalina* and *E. risdonii* stands and **ii)** on paired samples in and around the 'pure' *E. amygdalina* species transect in i). Cross types represented are: A(p), *E. amygdalina* from an adjacent pure species stand; A, *E. amygdalina*; AH, phenotype resembling an advanced generation backcross to *E. amygdalina* in the hybrid zone; H, intermediate F_1 hybrid phenotype in the hybrid zone; RH, phenotype resembling an advanced generation backcross in the hybrid zone; R, *E. risdonii* ; R(p), *E. risdonii* from an adjacent pure species stand. Significant differences at the 0.05 level, are indicated by different letters (see methods and Table 6.2).

i)



ii)



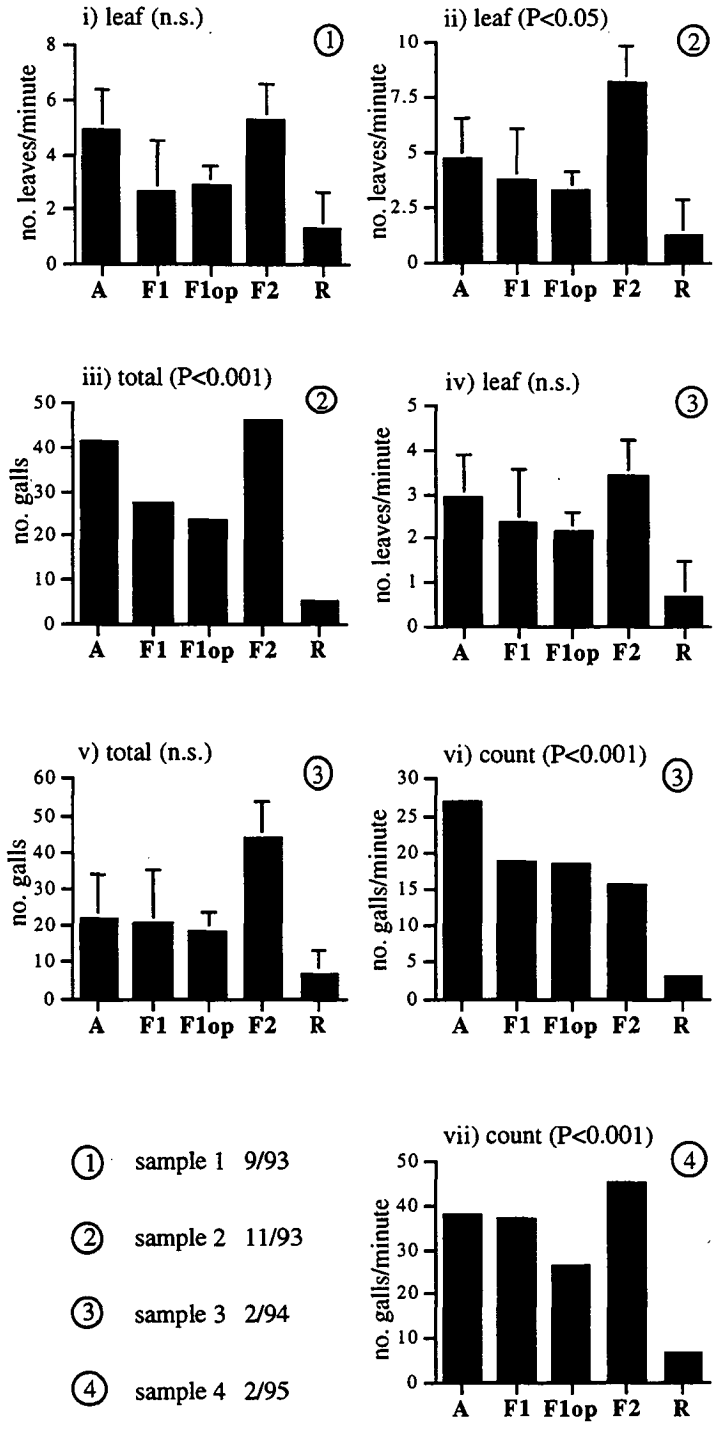


Figure 6.4. Cross type means for the different assessments of the hymenopterous gall 1 in the field trail (where relevant s.e. bars are indicated). Four different samples of the population in the trial were taken: sample 1 9/93; sample 2 11/93; sample 3 2/94 and sample 4, 2/95. Sample times are indicated by the numbers on the right of each graph. The three different methods of surveying the gall population used (see methods, Table 6.1) are indicated by either: count, for individual gall counts/minute; total, for whole tree gall counts and; leaf for a count of the number of leaves with galls/minute. The significance of comparisons between these cross types are given in Table 6.4.

Chapter 7

Oviposition preference and survival of the eucalypt weevil *Gonipterus scutellatus* on *Eucalyptus amygdalina, E. risdonii* and their hybrids

Introduction

The eucalypt weevil *Gonipterus scutellatus* (Gyllenhal) (Coleoptera: Curculionidae) has a wide host range in the genus *Eucalyptus* (Tooke 1955, Elliot and deLittle 1984). *E. globulus* and *E. viminalis* are two of the most preferred host species, although *E. gunnii, E. macarthurii,* and *E. radiata* are also favoured hosts (Tooke 1955, Elliot and deLittle 1984, Richardson and Meakins 1986). Both the adults and larvae feed on the young expanding eucalypt foliage (Tooke 1955, Elliot and deLittle 1984). Larval damage is very characteristic, showing a characteristic tracking on the leaves (See Figure 7.1). *G. scutellatus* has caused extensive damage to plantations outside Australia, particularly to *E. viminalis* in South Africa (Tooke 1955, Richardson and Meakins 1986). Heavy damage causes bushy, stunted trees and tree death can occur if damage is severe (Elliot and deLittle 1984).

Weevil damage was found to be greater on *E. amygdalina x E. risdonii* hybrids than pure species, in an experimental field trial (see Chapter 5, Figure 5.4ii). This unequal distribution of damage was not found to be related to the leaf oil or wax content of the foliage (see Chapter 5). Further investigation into the cause of the weevil damage was therefore needed to ascertain possible mechanisms behind the preferential browsing of the hybrids in the field trial. This study aimed to investigate the oviposition preference and survival of weevils on the different cross types within the trial (including *E. amygdalina, E. risdonii* and hybrids between these species), in an attempt to determine a possible cause of the different damage levels on different cross types noted previously.

This insect was chosen for two main reasons. Firstly it was shown to preferentially damage the hybrid phenotypes (F_1, F_2 and F_1 op, see Chapter 5). Secondly, there was a large population of the insect within the hybrid trial. In particular, there was a large number of weevils ovipositing throughout the trial. While other insects were present, no others were ovipositing in numbers large enough to allow an effective study of the cause of preferential damage on phenotypes within the trial.

Materials and methods

Experimental field trial

A subset of the *E. amygdalina x E. risdonii* experimental field trial (detailed in Chapter 5) was used for this experiment. This corresponded with the subset used for the analysis of leaf chemistry and leaf toughness (see Chapter 5 for details). Cross types included in the subset were *E. risdonii, E. amygdalina*, their F_1 hybrid, the reciprocal F_1 hybrid cross, and a single F_2 hybrid family. Most cross types were represented by a single family only, except for the F_1 hybrids, which included the *E. amygdalina x E. risdonii* hybrid family, and the reciprocal cross (*E. risdonii x E. amygdalina*), see Chapter 5. All the F_1 hybrids and the pure parents *E. risdonii* and *E. amygdalina* were directly related. While the F_2 family was obtained from the same area as the other cross types, it was not directly related.

Oviposition

A number of eggs were collected from the natural population in the trial at Copping on 31/10/94. The egg capsules were dissected and the number of eggs contained within each capsule was determined. The egg capsules were all a distinctive shape (Figure 7.1, Tooke 1955) and the number of 'bumps' on the upper capsule surface was found to equal the number of eggs within the capsule. This could easily be determined with the naked eye. Problems with determining the number of eggs within egg capsules would be encountered when egg capsules were more than one layer. Egg capsules with more than one layer were not noted in any that were collected. Further field observations on 3/11/94 determined that it did not appear that multi-layered eggs were present in the trial. This fact enabled accurate counts of oviposition to be obtained within the trial. Hence it was proposed to count the number of egg cases and the total number of eggs per individual tree for a subset of cross types within the field trial in order to determine oviposition preference of *G. scutellatus*.

The total number of eggs, the number of egg capsules (hereafter referred to as capsules) and the number of eggs in each capsule were determined for each tree from the subset in the field trial on the tenth of November 1994.

Survival

The survival of the eggs and larvae hatched from these eggs surveyed was followed each week on the same day for the nine weeks following the tenth of November. The survey included when the eggs hatched, whether they remained unhatched for the whole period of time or whether they were lost from unknown causes. Unknown causes included being washed off the leaf by rain, which was quite common, or being lost through abrasion in high winds. The larvae always emerge from the egg

capsule by eating their way through the leaf, so that egg capsules that had hatched were clearly identifiable (see Tooke 1955). It was also noted if eggs were lost due to the leaf they were oviposited on being lost, or due to predation or competition. Partially or wholly eaten remains of eggs and egg capsules were clearly identifiable and subsequently classified as being preyed upon. Eggs that died from dessication due to the surrounding tissue being partially or wholly eaten by other insects were subsequently classified as lost due to competition. In a very small number of instances the tags locating the eggs were lost and this was documented separately. The number of larvae present was determined in the early instars for each individual egg case for the first few weeks of the survey. However, due to larvae movement, the number of larvae present was later summed over the whole tree. No population or biology studies of *Gonipterus scutellatus* were attempted, so the actual instar of the larvae could not be confidently noted in addition to the other information. However, the size of the last instar larvae made it possible to note when larvae reached this stage of their life cycle. This was useful in that it gave a good indication of the possible number of larvae which may have successfully reached the adult stage of the life cycle (although mortality would also occur through pupation).

It is important to note that some oviposition was still occurring in the trial over the period of the survey. However, all eggs found on trees after the initial labelling were removed.

Egg parasitoids

A few extra eggs were collected from trees throughout the trial, where oviposition had occurred on the marked trees in the subset, in December 1995. The number of egg parasitoids that emerged from these eggs and the number of parasite species were determined. Most of the eggs collected were from hybrid plants, as this was the cross type that also had the greatest oviposition. Preliminary identifications of egg parasitoids were undertaken primarily by Dr. Anthony Clarke of the Cooperative Research Centre for Temperate Hardwood Forestry, Hobart.

Analysis

Family residuals for the number of eggs, the number of egg capsules and the number of eggs per capsule were unable to be normalised by any standard transformation. Data were therefore split into categories. These categories were: numbers of eggs, eggs = 0, 20^3eggs>0, 100^3eggs>20, eggs>100; numbers of egg capsules, capsules= 0, 0<capsules<30, capsules >30; numbers of eggs per capsule, eggs/capsule ^{2}1, 1<eggs/capsule<3, eggs/capsule^{3}3. Data were then analysed using the CATMOD procedure of SAS using cumulative logits (for ordinal data) with the response function and cross type effect in the model (SAS 1992).

The percentage eggs gone due to unknown causes (%gone), percentage eggs unhatched and percentage larvae surviving relative to the number of eggs laid, were calculated for each individual tree. Family residuals of these data were not significantly different from normal and were therefore analysed using the GLM procedure of SAS (SAS 1992) and the simple models:

$$\% \text{ gone} = \text{cross} + \text{error}$$
$$\%\text{unhatched} = \text{cross} + \text{error}$$
$$\%\text{survival} = \text{cross} + \text{error}$$

The F_1 hybrids were the only cross type in the plant material used that had greater than one family. Hence, in the overall analysis, family within cross type was not used in the model. Pairwise comparisons were undertaken between cross types using the contrast statement in GLM.

The actual number of larvae present was also determined for each cross type at each week of sampling. Family residuals for this trait were not able to be normalised by any standard transformation. Data were therefore divided into the following categories: larvae = 0, 0<larvae<5, larvae≥5 and analysed using the CATMOD procedure in SAS as previously described.

Arithmetic means of all traits investigated were obtained for graphical presentation.

Results

Oviposition

Hybrids within the trial were clearly favoured for oviposition in terms of both the number of capsules laid and the total number of eggs when compared with the pure species *E. amygdalina* (A) and *E. risdonii* (R) ($P \leq 0.001$, Table 7.1, Figure 7.2). F_1 hybrids and F_2 hybrids had over 11 times greater oviposition than both *E. amygdalina* and *E. risdonii*. No oviposition preference between *E. amygdalina* and *E. risdonii* was apparent, since both capsules and total number of eggs was not significantly different for these species ($P= 0.279$ and $P=0.311$ respectively, see Table 7.1).

The number of eggs per capsule laid was significantly greater on both the F_1 and F_2 hybrids when compared with *E. risdonii* ($P= 0.013, 0.004$; Figure 7.1 iii, Table 7.1 iii). The number of eggs per capsule on *E. amygdalina* was also lower than the hybrids, however this difference was not significant ($P=0.217$ for F_1, $P= 0.087$ for

F_2). The number of eggs per egg capsule was lowest on *E. risdonii* although it was not significantly lower than *E. amygdalina* (P= 0.136).

Survival

There were virtually no larvae on *E. amygdalina* (Figure 7.3). The number of larvae present on the F_1 and F_2 hybrids was significantly higher than on *E. risdonii* and *E. amygdalina* (P<0.05, week 2 and 3). The percentage larvae present relative to number of eggs laid, showed significant cross type differences at week 1 and 3 (P=0.046, P=0.001, GLM). Early in the sampling period, both the percentage larvae present and real larvae numbers were therefore greater on the hybrids than the pure parents.

No eggs oviposited on *E. risdonii* were lost due to unknown factors (gone, Figure 7.5 i). However, the number of eggs oviposited on both *E. risdonii* and *E. amygdalina* was low (Figure 7.2 i), and it would be difficult to generalise about eggs lost on these crosses *per se*. A significant difference between cross types was obtained for the percentage eggs gone after week 2 (P= 0.019). *E. amygdalina* had significantly greater egg loss than *E. risdonii* at week 2 (P=0.002). All cross types lost significantly higher percentage of eggs to unknown factors (% eggs gone, $P \leq 0.017$ at week 3) than *E. risdonii* after and including week 3, although they were not significantly different from each other (e.g. at week 3, $P \geq 0.616$). Cross types other than *E. risdonii* had similar loss of eggs (% eggs gone), reaching a maximum at approximately 24 to 28%.

The numbers of unhatched eggs were higher on the F_1 and F_2 hybrids when compared with *E. risdonii* and *E. amygdalina* (P≤0.000, week 2), although this was not always significant (e.g. week 9, F_1 vs. R, P = 0.09). A significant difference between cross types was noted for all weeks of sampling (P<0.006 for weeks 1-8, P=0.022 week 9), with the F_1 hybrids consistently having significantly more unhatched eggs than *E. risdonii* ($P \leq 0.007$). The F_2 hybrids had more unhatched eggs than the pure species for most weeks, however this difference was never significant.

Eggs lost in the field from other unknown causes was found to be greatest on the F_1 and F_2 hybrids (Figure 7.5, e.g. P≤0.05, week 5). The major cause of losses in this category was egg loss from leaf abscission or leaf loss. However, competition with other insects, causing the loss or death of eggs was also an important cause of egg loss. Very few eggs were lost from predation.

Egg parasitoids

Five taxa were preliminary identified after emerging from *G. scutellatus* eggs. These included *Anaphes nitens, A. tasminae, A. inexpectatus* and two pteromalid species (all Hymenoptera). From the 16 F_2 samples and 24 F_1 hybrid samples, 12 and 46 of these taxa were collected on these cross types respectively. 86% of the pteromalid species emerged from weevil eggs collected on F_1 hybrids and only 14% from the F_2 hybrids. No hymenopteran taxa emerged from the small number of eggs collected from *E. amygdalina* (1 sample) or *E. risdonii* (2 samples). Although results suggest that parasitism is potentially higher on the F_1 compared with the F_2 hybrids, these results are only preliminary and need to be verified from further egg collections.

Discussion

Damage caused by larvae of the gum tree weevil *Gonipterus scutellatus* was previously found to be preferentially distributed on the hybrids between *E. amygdalina* and *E. risdonii* (see Chapter 5). It appears that the difference in larval damage between cross types is largely due to a high oviposition preference for the hybrid cross types. Tooke (1955) suggested that eucalypt species with low levels of cineole were the most resistant to *G. scutellatus* attack. *E. risdonii* has higher levels of cineole than *E. amygdalina* (Li *et al.* 1994). Weevil damage was not found to correlate with the cineole content in the segregating F_2 population in Chapter 5. This result along with the fact that no difference in oviposition between the pure *E. amygdalina* and *E. risdonii* cross types was found, suggested that cineole was not the primary cause of host choice for *G. scutellatus.* However, it is possible that the hybrid phenotypes may have a disrupted or diluted resistance mechanism involving essential levels which make them more attractive for oviposition (see Whitham *et al.* 1994).

Richardson and Meakins (1986) suggest that narrow-leaf morphology may be closely associated with susceptibility to *Gonipterus* attack. *E. amygdalina* leaves are narrow lanceolate to linear with a lamina of 7-12cm., whereas *E. risdonii* leaves are ovate and broad, becoming connate (Chippendale 1988). It is possible that the very narrow leaves of *E. amygdalina* are too narrow for effective feeding, whereas the intermediate leaf shape of the hybrids provide the preferred leaf shape for oviposition cues.

Eggs that were oviposited on the different cross types were found to be lost primarily from being washed off in rainstorms or lost from mechanical abrasion caused by strong wind (referred to as 'other' causes). Rain and wind have previously been quoted as a major cause of egg loss, particularly for young eggs (Tooke 1955). A large proportion of eggs remained unhatched, with % of eggs still unhatched on F_1

hybrids after nine weeks of observation. A larger percentage of eggs remained unhatched on the hybrids than on the pure species. Egg losses through predation and competition were relatively low.

The number of larvae, percentage of unhatched eggs and eggs lost due to unknown causes were all greatest on the hybrid cross types in early weeks of sampling. It appears that the survival of larvae relative to the number of eggs laid was better on the hybrids than on the pure species. However, comparisons between cross types at this level are difficult due to the low oviposition on the pure species *per se.* Trends indicate that larval survival is better on the hybrids, that they have greater numbers of unhatched eggs, however further work including larval transferral experiments are needed before this can be confidently confirmed.

The susceptibility of hybrids has been found to extend to other trophic levels (Martinsen and Whitham 1993). *G. scutellatus* is known to be heavily parasitised by *A. nitens* (Huber and Prinsloo 1990). There was therefore a distinct possibility of differential parasitism of weevil eggs on the different cross types within the field trial. After initial surveys of weevil eggs, a number of emergent hymenopteran taxa were preliminarily identified and included *Anaphes nitens, A. tasminae* and *A. inexpectatus* and two pteromalids. The numbers of hymenopteran taxa that emerged from eggs collected on the F_1 hybrids was greater than on the F_2 hybrids, suggesting that F_1 hybrids may have greater parasitism levels. However, oviposition was too low later in the season after initial identifications to enable a more thorough investigation into the levels of parasitism on the different cross types. It is possible that the larger number of unhatched eggs found on the hybrid phenotypes may to some extent represent higher parasitism. Further work is needed to determine if this was the case.

Table 7.1. Oviposition on *E. amygdalina* (A), *E. risdonii* (R), F_1 hybrids (F_1) and a single F_2 family (F_2). Probabilities obtained from pairwise comparisons of i) the number of eggs, ii) the number of capsules, and iii) the number of eggs per capsule. All contrasts were obtained using the CATMOD procedure of SAS (see methods).

i) number of eggs

	A	F_1	F_2
F_1	0.001		
F_2	0.001	0.928	
R	0.311	0.000	0.000

ii) number of capsules

	A	F_1	F_2
F_1	0.001		
F_2	0.000	0.807	
R	0.279	0.000	0.000

iii) number of eggs per capsule

	A	F_1	F_2
F_1	0.217		
F_2	0.087	0.666	
R	0.136	0.013	0.004

Figure 7.1. i) Damage by *Gonipterus scutellatus* causes characteristic tracking in the leaf lamina. ii) Young larvae and egg capsules (e) on a shoot of *Eucalyptus*.

i)

ii)

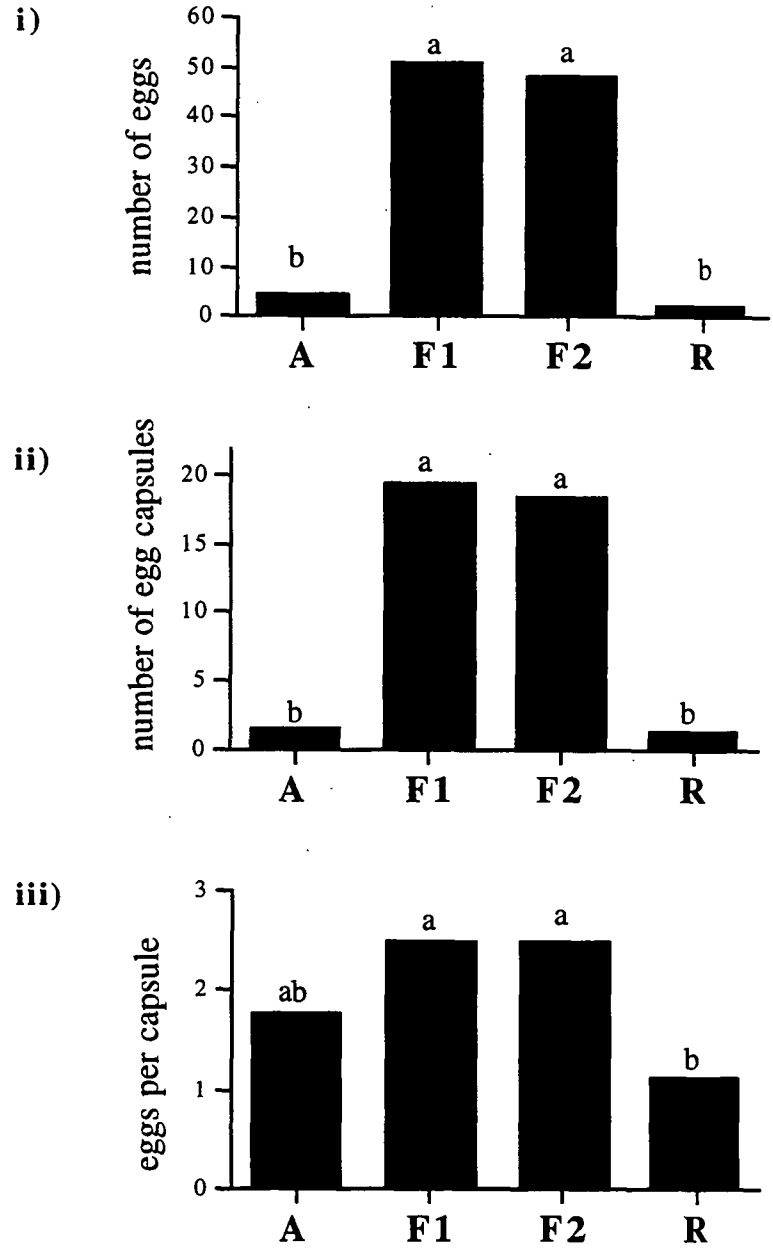


Figure 7.2. Oviposition preference on *E. amygdalina* (A), *E. risdonii* (R), first generation hybrids (F_1) and a single second generation hybrid family (F_2).Figures show i) The number of eggs on each cross type, ii) the number of egg capsules and iii) the number of eggs per capsule oviposited on these cross types in the field trial. Significant differences at the 0.05 level are represented by different letters above the cross types (see methods).

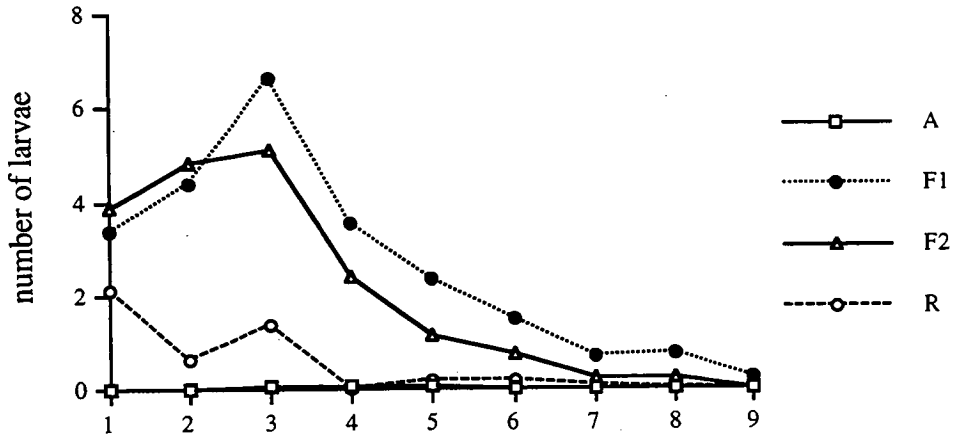


Figure 7.3. The mean number of larvae on each cross type over the nine week sampling period. Cross types are: *E. amygdalina* (A), *E. risdonii* (R), first generation hybrids (F_1) and a single second generation hybrid family (F_2).

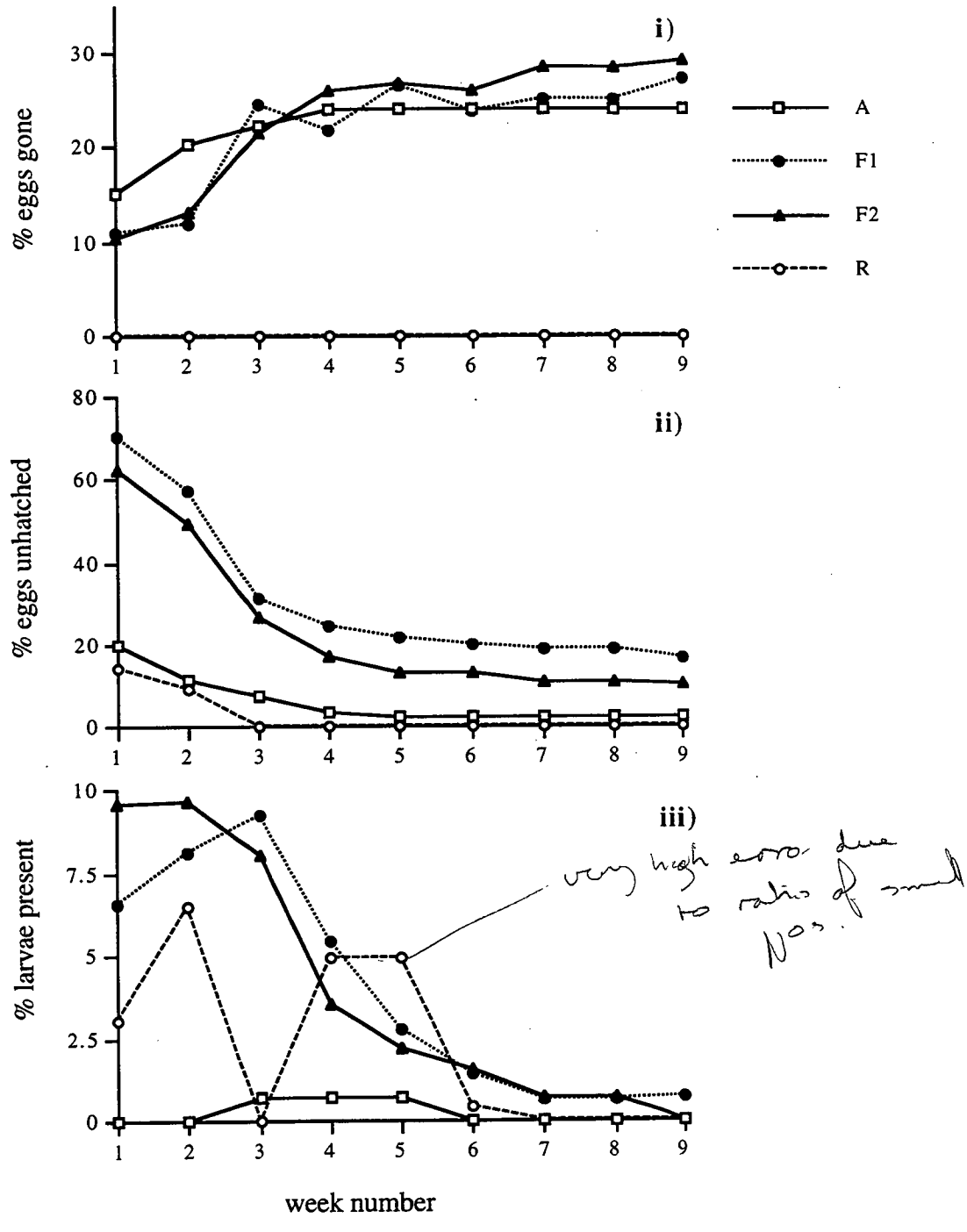
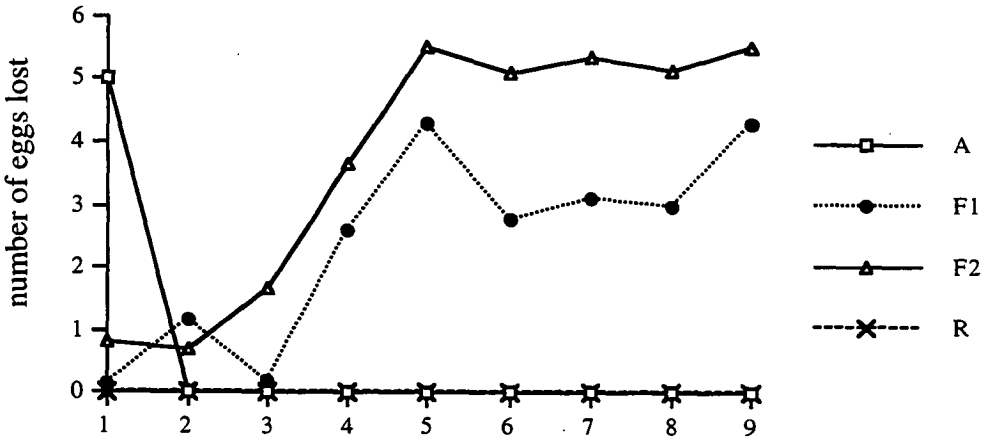


Figure 7.4. Fate of eggs and larvae oviposited on *E. amygdalina* (A), *E. risdonii* (R), first generation hybrids (F_1) and a single second generation hybrid family (F_2). Mean percentage eggs that were i) gone from unknown causes, ii) unhatched and iii) the mean percentage larvae surviving relative to the number of eggs laid are given for each cross type.

i)



ii)

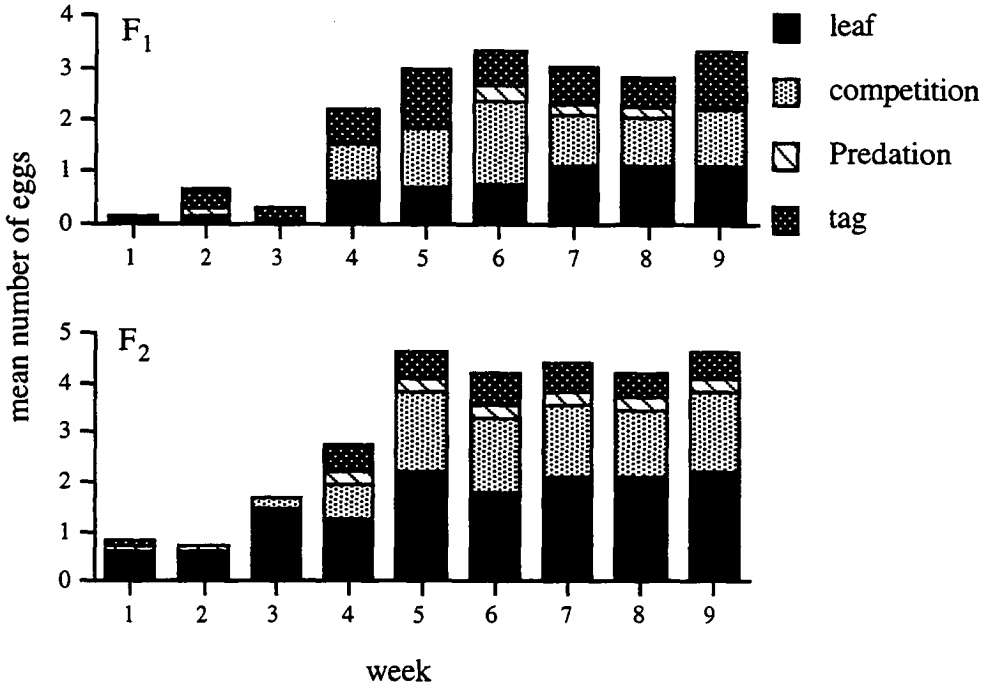


Figure 7.5. The mean number of eggs lost by other causes on A, F_1 F_2 and R cross types. Other causes are separated for F_1 and F_2 cross types in ii) and include competition, where eggs remained unhatched because of competition from other insects (e.g. where the leaf was eaten), predation, where eggs were eaten, and losses that were unaccounted for: loss of the leaf eggs were oviposited on (leaf) and loss of tags labelling eggs (tag). See previous legend for cross type codes.

Chapter 8

General Conclusions

This thesis aimed to examine the genetic nature of hybrid susceptibility, previously studied in natural hybrid zones between different tree species (e.g. in *Populus* Whitham 1989, *Quercus* Boecklen and Spellenberg 1990, Aguilar and Boecklen, *Betula* Hanhimäki *et al.* 1994). One of the main disadvantages of previous studies of natural hybrid zones is that hybrid identification was based largely on morphology alone and environmental effects such as stress and spatial grouping of phenotypes can not be excluded from contributing to the observed responses. A full understanding of hybrid susceptibility therefore depends on comparing the response of plants of known pedigree and genotype in controlled or common environments (Fritz *et al.* 1994). The present study is one of the first detailed assessments of hybrid susceptibility to pests using hybrid material of known pedigrees growing with parental phenotypes in a common environment (that is randomised field trials). Environmental effects are randomised at the cross types or family level and any differences in response patterns must, one way or another, have an underlying genetic basis.

A wide variety of responses to hybrids were observed in the individual insect, vertebrate and fungal taxa examined in this study. Fritz *et al.* (1994) proposed that the response of herbivores to hybrids could be classified into several categories. These included the additive response, where the resistance of hybrids did not differ from the mean resistance of the two parent species. The dominance response was upheld if hybrid resistance differed significantly from the mean resistance of both parents, but not from one of the parents. Hybrid susceptibility was implied when the hybrid had significantly higher herbivore success than both parent species. The resistance response was supported when hybrids had significantly lower herbivore success than both parent species.

In the present study there was clearly a general trend for pests to show additive or dominance responses to hybrids when there was a marked difference in the susceptibility of the host parent species. For example, in Chapter 3, *E. gunnii* and *E. morrisbyi* were highly susceptible to vertebrate browsing by *Trichosurus vulpecula* whereas *E. nitens* and *E. globulus* were relatively resistant. In all cases the first generation (F_1) hybrids between resistant and susceptible species had intermediate levels of browsing although the degree of dominance between one or other species

varied. In Chapter 4, *E. globulus* and *E. cordata* were susceptible to *Uraba lugens*, and hybrids with less susceptible species generally had intermediate damage levels. Furthermore, in Chapter 5, the majority of species specific insect taxa showed dominance responses to F_1 hybrids between *E. amygdalina x E. risdonii.*

In contrast, when hybrids were significantly more susceptible to a pest species than parental types, there was often little difference in the susceptibility of parental host species. This was shown in Chapter 5, where susceptible responses to *E. amygdalina x E. risdonii* hybrids were exhibited by all four generalist insect taxa. In Chapter 7, oviposition by the eucalypt weevil was clearly biased towards hybrid phenotypes, yet no significant difference was found between the parental species *E. amygdalina* and *E. risdonii.* Similarly, when there was no significant difference in the susceptibility of parent species to possum damage in Chapter 3, there was a trend for F_1 hybrids to be more susceptible (*E. ovata x E. globulus, E. nitens x E. globulus*), although this was only significant in one case. In the case of host susceptibility of *E. nitens* and *E. globulus* to *Mycosphaerella* leaf disease, discussed in Chapter 2, intra-specific differences were inherited in an additive manner. However, while parental species differed little in their susceptibility, inter-specific hybrids were generally more susceptible than the mid-parent value, and in some instances (juvenile foliage) more susceptible than the most susceptible parent species. Exceptions to these general trends were seen in Chapter 4, where qualitative assessments suggested that F_1 hybrids were more susceptible to chrysomelid damage than parental controls, regardless of the difference between parent species.

There are obviously complex interactions involved in ascertaining hybrid susceptibility in the field. While there may be a genetic basis for the susceptibility, this may be modified by interactions with both the biotic (e.g. competitors, predators and alternative host species), and abiotic environment. For example, there are suggestions in Chapter 3, that the response of possums to *E. nitens x E. globulus* hybrids depended on the availability of alternative host sources. The genetic susceptibility of the F_1s appeared to be manifest only in the absence of more attractive host species (e.g. *E. morrisbyi E. gunnii*). In Chapter 2, complex interactions between growth, vegetative phase change and the severity of *Mycosphaerella* leaf disease were detected. Over all cross types, disease severity was greater on juvenile foliage than adult foliage and the relative susceptibility of hybrids changed at different ontogenetic stages. Further complexities were evident in the distribution of a key hymenopteran leaf gall examined on natural and artificial hybrids between *E. amygdalina* and *E. risdonii* in Chapter 6. In one natural hybrid zone, this gall was concentrated on hybrid phenotypes and no species preferences were detected, a response which was stable over a four year period. However, in a

separate hybrid zone nearby where gall numbers were higher, marked differences in host species preference were detected and the hybrids exhibited a dominant response. Furthermore, host species preference (*E. amygdalina* versus *E. risdonii*) was completely reversed in the experimental field trial and the hybrids generally showed an intermediate response.

There were clearly many abiotic factors involved in determining the distribution of some taxa than just host genotype or phenotype alone. It was likely that interactions with the immediate environment both in the natural hybrid zones and in the field trial situations were important. Further investigation on the effects of environmental variables on the levels of stress and how they were correlated with pest loads would be advantageous. In particular stress caused by water, light and nutrient availability, and temperature are important in determining eucalypt distribution (Davidson and Reid 1985, Davidson and Reid 1987, Davidson and Reid 1989, Kirkpatrick and Marks 1985) and these factors could therefore be important in lowering host resistance to pests.

Whitham *et al.* (1992, 1994) had previously demonstrated increased richness of insect species taxa on hybrids in a natural hybrid zone between *E. amygdalina* and *E. risdonii.* Furthermore, Whitham *et al.* (1992, 1994) argued that hybrid zones may be of high conservation value and important for the conservation of biodiversity. The present study clearly demonstrated a genetic basis to the increase in species richness on hybrids. The pattern of species richness observed in the natural hybrid zone was repeated following three years of natural colonisation of an experimental field trial. Hybrids were susceptible to a greater number of insect taxa and most insect taxa exhibited either an additive or susceptible response to the hybrids. No cases of hybrid resistance were detected.

Genetic susceptibility of hybrids to pests has been attributed to the breakdown of co-adapted gene sequences following genetic recombination in advanced generation hybrids (Whitham *et al.* 1994). If this were the case, the susceptibility of F_2 and advanced generation hybrids would be greater than F_1 hybrids. However, this was not the case for the hybrids between *E. amygdalina* and *E. risdonii.* The F_1 hybrids were susceptible to a greater number of insect taxa than advanced generation hybrids. In addition, the F_1 hybrids were equivalent or more susceptible than advanced generation hybrids to individual insect taxa. Such susceptibility may have been due to the dilution of resistance genes where different genetic mechanisms for resistance from parental species were inherited in an additive or recessive manner and gene products were reduced below a threshold level.

The mechanisms involved in host susceptibility, including host choice, were examined in Chapters 5 and 7. Preferential grazing of hybrids by larvae of the eucalypt weevil (*Gonipterus scutellatus*) appeared be related to selective oviposition on these phenotypes by the adults. However, the direct cause of the oviposition choice was not ascertained although possible mechanisms involved were investigated (leaf toughness and leaf oil content). One advantage of the present study was the availability of a large, segregating F_2 family. This enabled associations between insect loads and host characteristics to be examined on relatively randomised genetic background which would minimise the chance of spurious associations being detected. Leaf toughness and leaf oils have been implicated in host choice and insect survival in eucalypts in some instances (e.g. Edwards and Wanjura 1990, Ohmart 1991, Edwards *et al.* 1993, Li 1993, Floyd and Farrow 1994). However, in the present study only one association was detected. The distribution of a hymenopterous leaf gall was virtually limited to F_2 trees where no eudesmols were detected in the leaf oil analysis. While eudesmols have been implicated as antimite agents (Morita and Yatagai 1994), further research is required to verify this association and to determine whether it was a direct or indirect effect.

The susceptibility of hybrids has a number of important consequences for both plant and insect populations. For plant populations, the reduced fitness caused by increased susceptibility to pests (e.g. Drake 1975, Drake 1981, Potts 1986, Whithan *et al.* 1994) is likely to contribute to maintaining host species integrity and the impact of pests on plant fitness requires further study. Floate and Whitham (1993) argue that hybrids may morphologically, genetically and spatially bridge gaps between parental species and allow herbivores to shift hosts in a series of gradual steps. In such cases, host shifting is more likely to occur in the presence of hybrids than in their absence (Floate and Whitham 1993). High insect loads were previously observed on parental phenotypes in the hybrid zone between *E. amygdalina* and *E. risdonii* than in pure species stands. However, in Chapter 6 similar results were also found for pure parental types at a boundary where hybrids were rare arguing against hybrids being the main cause of this pattern. Whitham (1989) also suggested that hybrids and hybrid zones may act as refugia for insect species when population numbers are low. This concept was clearly supported in Chapter 6 where the hymenopteran gall surveyed was concentrated on isolated hybrids within the pure *E. amygdalina* forest. The population density of the gall was low in the pure *E. amygdalina* forests and hybrids appeared to be acting as 'habitat islands', potentially providing stepping-stone links between favourable host populations.

The genetic control of susceptibility to insect and fungal taxa is a key issue for both hybrid and pure species breeding, with genetic gain from selection being largely

determined by the extent to which susceptibility is heritable. Within populations, heritability estimates for damage on controlled cross progeny ranged from low to moderately high (0.00 to 0.56). The highest heritabilities in pure species progeny were obtained for possum damage on *E. nitens* (0.56). Individual narrow-sense heritabilities obtained for possum damage were high for *E. nitens* and comparable with estimates obtained previously (0.30 and 0.38, Cannon 1993). Heritabilities obtained for *E. globulus* were low within a provenance, although significant differences between provenances were obtained. The proportion of dominance variation was very high in *E. nitens* controlled crosses, and at least as high as heritability estimates determined for the intra-provenance crosses of *E. globulus*. This suggested that only a few genes may have been controlling the genetic variation in possum damage in *E. nitens* in particular. It was apparent that there was sufficient genetic variation for breeding for resistance in this trait, both within a provenance (*E. nitens*) and between provenances (*E. globulus*).

Similarly, genetic variation was sufficient in *Mycosphaerella* disease severity was sufficient to enable gain to be made through breeding. Within species, in *E. globulus*, disease was more severe on one provenance, Taranna, than on the other provenance, King Island. Within populations of *E. globulus* and *E. nitens*, the narrow-sense heritabilities for *Mycosphaerella* disease severity were low to moderate (0.004-0.506), but were consistently higher for adult than for juvenile foliage. Open-pollinated progeny are often used to estimate parental breeding values in *Eucalyptus*. However, while inbreeding depression appears to result in a poor estimates of genetic parameters from open-pollinated progeny for growth traits (Hodge *et al.* 1995), Chapter 2 showed that for *Mycosphaerella* disease severity, breeding values and heritabilities estimated from open-pollinated progeny were similar to estimates obtained from controlled crosses involving the same parents. In contrast, the association between parental breeding values estimated in hybrid and pure species combination were poor and predicting hybrid susceptibility from pure parent performance was not possible.

Overall, while hybrids elicited an intermediate response to most individual pest taxa, the fact that more taxa were found on hybrid phenotypes or genotypes *per se* meant that it is highly likely that eucalypt hybrids will have the greatest overall damage from the collective effects of many pest taxa. Only at the individual or family level were cases of hybrid resistance found in the present study (e.g. chrysomelid damage Chapter 4). However, the successful exploitation of hybrids in *Eucalyptus* (e.g. *E. grandis x E. camaldulensis* and *E. tereticornis x E. urophylla* in Africa, Van Wyk *et al.* 1988; *E. grandis x E. urophylla* in Brazil, Campinhos and Ikemori 1989) and other genera (e.g. in *Pinus* Nikles 1991, Blada 1994; *Populus* Wu 1994, Rajora *et al.*

1994) suggests that with intensive selection it may be possible to obtain hybrids that are not highly susceptible to pests. It is therefore conceivable that inter-specific hybrids will be useful for resistance breeding, particularly if variation at an individual or family level is exploited.

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